

09/989974

(FILE 'REGISTRY' ENTERED AT 15:04:35 ON 24 OCT 2003)

L1 E MERCURY/CN  
5 S E3 OR E8 OR E9-E11  
E PLATINUM/CN  
L2 1 S E3  
E IODINE/CN  
L3 6 S E3-E9  
E IRON/CN  
L4 7 S E3 OR E14-E19  
E SELENIUM/CN  
L5 11 S E3-13  
L6 30 S L1 OR L2 OR L3 OR L4 OR L5  
E SELENOMETHIONINE/CN  
L7 3 S E3-E4  
E SELENOSYSTEINE/CN 5  
E SELENOCYSTEINE/CN 5  
L8 1 S E3  
L9 4 S L7 OR L8

-key terms

FILE 'HCAPLUS' ENTERED AT 15:07:25 ON 24 OCT 2003

L7 3 SEA FILE=REGISTRY ABB=ON PLU=ON (SELENOMETHIONINE/CN  
OR "SELENOMETHIONINE (75SE)"/CN)  
L8 1 SEA FILE=REGISTRY ABB=ON PLU=ON SELENOCYSTEINE/CN  
L9 4 SEA FILE=REGISTRY ABB=ON PLU=ON L7 OR L8  
L14 1573 SEA FILE=HCAPLUS ABB=ON PLU=ON (PROTEIN OR POLYPROTEIN  
OR POLYPEPTIDE OR PEPTIDE) AND (L9 OR SELENOMETHIONINE  
OR SELENO!YSTEIN? OR (SE OR SELENO) (W) (METHIONINE OR MET  
OR CYSTEIN? OR CYS OR SYSTEIN? OR SYS) OR SEMET OR SECYS  
OR SESYS)  
L15 13 SEA FILE=HCAPLUS ABB=ON PLU=ON L14 AND (COLI OR  
BACTERI## OR YEAST) (10A) (EXTRACT? OR EXT##)

L15 ANSWER 1 OF 13 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2002:806817 HCAPLUS

DOCUMENT NUMBER: 138:201238

TITLE: Development of new analytical methods for  
selenium speciation in selenium-enriched yeast  
material

AUTHOR(S): Chassaing, H.; Chery, C. C.; Bordin, G.;  
Rodriguez, A. R.

CORPORATE SOURCE: IRMM, European Commission-Joint Research Centre,  
Geel, B-2440, Belg.

SOURCE: Journal of Chromatography, A (2002), 976(1-2),  
409-422

CODEN: JCRAEY; ISSN: 0021-9673

PUBLISHER: Elsevier Science B.V.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A sequential extraction allowing the discrimination of water-soluble and non-soluble selenium fractions has been developed to evaluate the availability of selenium (Se) in an Se-enriched yeast candidate reference material. The fractionation of selenium-containing compds. in the exts. was achieved on preparative grade Superdex 75 and 200 columns. It showed that water-soluble selenium is present in several fractions with a large mass distribution. Low-mol.- ( $\leq 10,000$ ) and high-mol.-mass selenocompounds (range 10,000-100,000) were considered sep. for further expts. The anal. approach for low-mol.-mass selenocompounds was based on anion-exchange HPLC with

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online inductively coupled plasma (ICP) MS for quant. anal. Selenocystine, **selenomethionine**, selenite and selenate were quantified in the fractions isolated in preparative chromatog. The study revealed the existence of various unidentified Se species in yeast material. The Se-containing **proteins** in the yeast material have been further separated and selenium quantified by the combination of gel electrophoresis and electrothermal vaporization-ICP-MS. This new approach allows the separation of the **proteins** with high resolution by sodium dodecylsulfate-PAGE and the sensitive determination of selenium in the **protein** bands.

IT 3211-76-5, **Selenomethionine**

RL: ANT (Analyte); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study)

(anal. of selenium compds. in selenium-enriched yeast material)

REFERENCE COUNT: 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 2 OF 13 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2002:704578 HCAPLUS

DOCUMENT NUMBER: 137:212639

TITLE: Cell-free synthesis of heavy atom-containing **proteins** for x-ray crystallography structural analysis

INVENTOR(S): Nunokawa, Emi; Kikawa, Takanori; Yabuki, Takashi; Yokoyama, Shigeyuki

PATENT ASSIGNEE(S): Institute of Physical and Chemical Research, Japan

SOURCE: Jpn. Kokai Tokkyo Koho, 10 pp. CODEN: JKXXAF

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2002262867	A2	20020917	JP 2001-65799	20010308
US 2002168705	A1	20021114	US 2001-989974	20011120

PRIORITY APPLN. INFO.: JP 2001-65799 A 20010308

AB A method for large-scale cell-free synthesis of heavy atom-containing **proteins** suitable for x-ray crystallog. structural anal. using dialysis, is disclosed. Cell **extract** of *E. coli*, hyperthermophilic archaeon, or **yeast**, is used. It also includes ATP regeneration system, macromol. adsorbent, and reducing agent. Creatine kinase and creatine phosphate are used for ATP regeneration. Amino acids containing mercury, platinum, iodine, iron, or selenium, such as **selenocysteine** or **selenomethionine**, are to be incorporated. Synthesis of **selenomethionine**-containing Ras **protein** by cell-free synthesis system, crystallization by hanging-drop vapor-diffusion method, and structural anal. by multiwavelength anomalous diffraction (MAD), are described. The three dimensional structure model produced was identical to those of unlabeled **proteins** produced in vivo and in cell-free system.

IT 3211-76-5, **Selenomethionine** 3614-08-2, **Selenocysteine**

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RL: BUU (Biological use, unclassified); BIOL (Biological study);  
USES (Uses)  
(cell-free synthesis of heavy atom-containing **proteins** for  
x-ray crystallog. structural anal.)

L15 ANSWER 3 OF 13 HCAPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 2002:414966 HCAPLUS  
DOCUMENT NUMBER: 137:89976  
TITLE: Crystal structure of the V-region of  
Streptococcus mutans antigen I/II at 2.4 Å  
resolution suggests a sugar preformed binding  
site  
AUTHOR(S): Troffer-Charlier, Nathalie; Ogier, Joelle;  
Moras, Dino; Cavarelli, Jean  
CORPORATE SOURCE: Institut National de la Sante et de la Recherche  
Medicale U424 Faculte de Chirurgie Dentaire,  
Universite Louis Pasteur, Strasbourg, 67085, Fr.  
SOURCE: Journal of Molecular Biology (2002), 318(1),  
179-188  
CODEN: JMOBAK; ISSN: 0022-2836  
PUBLISHER: Elsevier Science Ltd.  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Antigens I/II are large multifunctional adhesins from oral viridans streptococci that exert immunomodulatory effects on human cells and play important roles in inflammatory disorders. Among them, Streptococcus mutans plays a major role in the initiation of dental caries. The structure of the V-region (SrV+, residues 464-840) of the antigen I/II of S. mutans has been determined using the multiwavelength anomalous diffraction phasing technique with **seleno-methionine**-substituted recombinant **protein** and subsequently refined at 2.4 Å resolution. The crystal structure of SrV+ revealed a lectin-like fold that displays a putative preformed carbohydrate-binding site stabilized by a metal ion. Inhibition of this binding site may confer to humans a protection against dental caries and dissemination of the **bacteria** to **extra**-oral sites involved in life-threatening inflammatory diseases. This crystal structure constitutes a first step in understanding the structure-function relationship of antigens I/II and may help in delineating new preventive or therapeutic strategies against colonization of the host by oral streptococci.

REFERENCE COUNT: 49 THERE ARE 49 CITED REFERENCES AVAILABLE  
FOR THIS RECORD. ALL CITATIONS AVAILABLE  
IN THE RE FORMAT

L15 ANSWER 4 OF 13 HCAPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 2002:6280 HCAPLUS  
DOCUMENT NUMBER: 136:196388  
TITLE: Analysis of selenized yeast for selenium  
speciation by size-exclusion chromatography and  
capillary zone electrophoresis with inductively  
coupled plasma mass spectrometric detection  
(SEC-CZE-ICP-MS)  
AUTHOR(S): Mounicou, Sandra; McSheehy, Shona; Szpunar,  
Joanna; Potin-Gautier, Martine; Lobinski,  
Ryszard  
CORPORATE SOURCE: Group of Bio-inorganic Analytical Chemistry,

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SOURCE: Universite de Pau et des Pays de l'Adour, CNRS  
UMR 5034, Helioparc, Pau, F-64053, Fr.  
Journal of Analytical Atomic Spectrometry  
(2002), 17(1), 15-20  
CODEN: JASPE2; ISSN: 0267-9477

PUBLISHER: Royal Society of Chemistry

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A two-dimensional separation approach based on size-exclusion chromatog. (SEC) followed by capillary zone electrophoresis (CZE) is proposed for the mapping of seleno-compds. in aqueous **exts.** of selenized **yeast**. The coupling of CZE with ICP-MS via a self-aspirating total consumption micronebulizer was optimized for the separation of Se species. Selenate, selenite, selenocystine, **selenomethionine** and selenoethionine could be baseline separated at pH 10.5 using a 10 mM phosphate buffer containing 0.8 mM cetyltrimmonium bromide. Detection limits were 7-18 ng mL<sup>-1</sup> for a 20 nL injection. The CZE-ICP-MS anal. of a **yeast extract** demonstrated the presence of many Se species, which all migrated less rapidly than any of the stds. The following difficulties occurred during the CZE-ICP-MS anal. of the SEC fractions of the extract: the recovery of the high-mol. Se-species from the electrophoretic capillary, the presence of a large number of compds. in the medium-mol. weight fraction and the presence of a single intense peak for the low-mol. weight fraction. Proteolysis of the high- and medium-mol. weight fractions dramatically improved the recovery of Se species from the capillary, resulting in several peaks in the CZE-ICP-MS electropherograms.

IT **1464-42-2, Selenomethionine**  
RL: ANT (Analyte); ANST (Analytical study)  
(selenized yeast for selenium speciation by size-exclusion chromatog. and capillary zone electrophoresis with inductively coupled plasma mass spectrometric detection)

REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 5 OF 13 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2001:492044 HCAPLUS

DOCUMENT NUMBER: 136:227352

TITLE: Extraction of DNA from environmental sample and construction of mixed genomic DNA library

AUTHOR(S): Wang, Xiaobo; Tang, Yuqiu; Wang, Jinhua; Huang, Yixiu; Chen, Runsheng; Huang, Li

CORPORATE SOURCE: Department of Biochemistry & Molecular Biology, College of Life Science, Beijing University, Beijing, 100871, Peop. Rep. China

SOURCE: Weishengwu Xuebao (2001), 41(2), 133-140  
CODEN: WSHPA8; ISSN: 0001-6209

PUBLISHER: Kexue Chubanshe

DOCUMENT TYPE: Journal

LANGUAGE: Chinese

AB A method for extracting and purifying genomic DNA from environmental samples was introduced. The environmental sample was treated first by grinding and freezing/thawing and subsequently by SDS/proteinase K-based DNA extraction. The yields of purified DNA from three samples used in the study ranged from 2 to 16µg per g of dry sample. The mixed genomic DNA libraries for two of the environmental samples

were constructed by inserting restriction fragments (3-8 kb) of the purified DNAs into plasmid pUC18 and transforming E. coli DH5 $\alpha$  with the resultant plasmids. Approx. 103 to 104 insert-containing clones were obtained from 1 g of each sample. The clone libraries were analyzed by DNA sequencing and gene annotation. Among 20 randomly-selected clones, 14 contained an insert whose sequence had not been reported while the rest had an insert of either E.coli or vector origin. A search of sequence databases using the end sequences of each of the foreign inserts showed that each sequence was part of a gene encoding, in most cases, a predictable function.

L15 ANSWER 6 OF 13 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2001:305991 HCAPLUS

DOCUMENT NUMBER: 135:16309

TITLE: The use of radiofrequency glow discharge-mass spectrometry (rf-GD-MS) coupled to gas chromatography for the determination of selenoaminoacids in biological samples

AUTHOR(S): Montes Bayon, Maria; B'Hymer, Clayton; Ponce de Leon, Claudia A.; Caruso, Joseph A.

CORPORATE SOURCE: Department of Chemistry, University of Cincinnati, Cincinnati, OH, 45221-0172, USA

SOURCE: Journal of Analytical Atomic Spectrometry (2001), 16(5), 492-497

CODEN: JASPE2; ISSN: 0267-9477

PUBLISHER: Royal Society of Chemistry

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A radiofrequency glow discharge (rf-GD) has been used as an ion source for mass spectrometry using gas chromatog. as the sample introduction technique for the speciation of seleno amino acids. The use of helium as the plasma gas allowed the monitoring of selenium's major isotope (80Se), interfered by polyat. species when using Ar as the plasma gas. Optimization of the power and pressure of the source was undertaken to achieve the best sensitivity for selenium determination and detection limits of about 100 pg (as Se) have been obtained for the derivatized **Se-methionine**. Studying the cell pressure and power on the fragmentation patterns obtained for **Se-methionine** and Se-ethionine has also been undertaken to characterize the source. No significant variations were observed on the mass spectra under different operating conditions, although noticeable variation of the fragment intensities was observed. The system has been applied to the determination of

**Se-methionine** in selenium-enriched yeast

after enzymic **extraction** of the corresponding species using Proteinase K to cleave the **peptide** bonds. The results obtained showed a **Se-methionine** content of about 78% in the enriched yeast.

IT 1464-42-2, Selenomethionine

RL: ANT (Analyte); ANST (Analytical study)

(radiofrequency glow discharge-mass spectrometry (rf-GD-MS)

coupled to gas chromatog. used for determination of selenoaminoacids in biol. samples)

REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

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L15 ANSWER 7 OF 13 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 1999:217599 HCAPLUS  
DOCUMENT NUMBER: 131:55971  
TITLE: Sample preparation and HPLC separation  
approaches to speciation analysis of selenium in  
yeast by ICP-MS  
AUTHOR(S): Casiot, Corinne; Szpunar, Joanna; Lobinski,  
Ryszard; Potin-Gautier, Martin  
CORPORATE SOURCE: CNRS EP132, Bio-Inorganique et Environnement,  
Laboratoire de Chimie Analytique, Universite de  
Pau et de Pays de l'Adour, Pau, 64 053, Fr.  
SOURCE: Journal of Analytical Atomic Spectrometry  
(1999), 14(4), 645-650  
CODEN: JASPE2; ISSN: 0267-9477  
PUBLISHER: Royal Society of Chemistry  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Eight solid-liquid **extraction** procedures were evaluated for the recovery of selenium species from **yeast**. Speciation of Se in the exts. was characterized by different types of HPLC, including size-exclusion, anion-exchange and reversed-phase chromatog. with ICP-MS detection. The results obtained depended critically on the sample preparation procedure used. Leaching with water and with methanol led only to 10-20% recoveries of Se, split into eight compds., among which Se(IV) and **selenomethionine** could be identified. Leaching with pectinolytic enzymes released an addnl. 20% of **selenomethionine**. Leaching with SDS solution allowed the solubilization of a selenoprotein that accounted for .apprx.30% of the total Se present. Leaching with proteolytic enzymes led to recoveries of Se above 85%, the majority as **selenomethionine**. Hydrolysis of the yeast with tetramethylammonium hydroxide solubilized the sample completely but the Se species present were entirely degraded to **selenomethionine** and inorg. selenium. A sequential leaching procedure is proposed for the evaluation of selenium speciation in yeast without the need for a coupled technique.

IT 1464-42-2, **Selenomethionine**

RL: ANT (Analyte); BOC (Biological occurrence); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); OCCU (Occurrence)

(sample preparation and HPLC separation approaches to speciation anal. of selenium in yeast by ICP-MS)

REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE  
FOR THIS RECORD. ALL CITATIONS AVAILABLE  
IN THE RE FORMAT

L15 ANSWER 8 OF 13 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 1996:181344 HCAPLUS  
DOCUMENT NUMBER: 124:252080  
TITLE: Transcription of Escherichia coli tRNA<sup>sec</sup> gene  
by RNA polymerase II in Saccharomyces cerevisiae  
AUTHOR(S): Zhang, Miao-Hua; Liu, Jian-Hua; Jin, You-Xin  
CORPORATE SOURCE: Shanghai Inst. of Biochem., Chinese Academy of  
Sci., Shanghai, 200031, Peop. Rep. China  
SOURCE: Shengwu Huaxue Yu Shengwu Wuli Xuebao (1995),  
27(5), 529-36  
CODEN: SHWPAU; ISSN: 0582-9879  
PUBLISHER: Shanghai Kexue Jishu Chubanshe

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DOCUMENT TYPE: Journal  
LANGUAGE: Chinese

AB The **selenocysteine** tRNA (SelC) gene of E. coli was inserted into a secretory expression plasmid vector, PVT102U- $\alpha$ MFL, with adjusted reading frame of **protein** translation, and added stop codon at the downstream of the structure gene. Its expression was established in Saccharomyces cerevisiae. A 7-8kd band in SD culture media was shown by SDS-PAGE anal. to be consistent with the theor. value. The total RNA was **extracted** from **yeast** and Northern blot was done with a 5' end-labeled 21-mer oligonucleotide probe complementary to the T $\Psi$ C region of the tRNA<sup>sec</sup>. Besides one weak band there were two strong bands, one of which corresponded to 790 nts and the other 370 nts. It is suggested that the 790 nts band is the unprocessed precursor of the RNA polymerase II-mediated transcript, the 370 nts band in RNA mols. processed at the 5'-end ends but not the 3'-ends, and the weaker band is the mature tRNA of about 90 nts long. We concluded that RNA polymerase II could transcribed tRNA genes. Because of the low level of 3'-endonuclease in the yeast strain we used, the 3'-end processing of the precursors with long tails was slow.

L15 ANSWER 9 OF 13 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 1996:122975 HCAPLUS

DOCUMENT NUMBER: 124:212199

TITLE: Assessment of selenium chemical species in selenium yeast

AUTHOR(S): Yang, Linsheng; Hou, Shaofan; Li, Dezhu

CORPORATE SOURCE: Inst. Geography, Academia Sinica, Beijing, 100101, Peop. Rep. China

SOURCE: Zhongguo Yaoxue Zazhi (Beijing) (1995), 30(11), 680-2

CODEN: ZYZAEU; ISSN: 1001-2494

PUBLISHER: Zhongguo Yaouxuehui

DOCUMENT TYPE: Journal

LANGUAGE: Chinese

AB Se **yeast** was **extracted** by a tris-buffer-glycerol mixture, and the process was compared with a dialysis method. Total Se was 4000  $\mu$ g/g-yeast, and more than 50% of Se in the Se-yeast was in **protein** bound mols. as **selenomethionine**. The inorg. species accounted for 3%, and the free species 0.01%, indicating that the Se-yeast has development prospective.

L15 ANSWER 10 OF 13 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 1995:973164 HCAPLUS

DOCUMENT NUMBER: 124:81066

TITLE: Selenoamino acid speciation using HPLC-ETAAS following an enzymic hydrolysis of selenoprotein

AUTHOR(S): Gilon, N.; Astruc, A.; Astruc, M.; Potin-Gautier, M.

CORPORATE SOURCE: Laboratoire de Chimie Analytique, Univ. de Pau et des Pays de l'Adour, PAU, 64000, Fr.

SOURCE: Applied Organometallic Chemistry (1995), 9(7), 623-8

CODEN: AOCHEX; ISSN: 0268-2605

PUBLISHER: Wiley

DOCUMENT TYPE: Journal

LANGUAGE: English

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AB A HPLC-electrothermal atomic absorption spectroscopy (HPLC-ETAAS) hyphenated technique was used for the determination of seleno compds. present in a selenium-enriched yeast. Conditions were optimized for the separation and quantification of the selenoamino acids, selenocystine and **selenomethionine**, in the presence of other compds. The separation was achieved by ion-pairing chromatog. using sodium heptane sulfonate as the anionic counterion. Online detection was carried out using electrothermal atomic absorption with palladium(II) as a matrix modifier. Different **extraction** procedures were tested on a selenium-enriched **yeast**. A 92% recovery of the total selenium present in the material was obtained. Attempts to evaluate selenium speciation were carried out; **selenomethionine** and selenocystine were identified as the major components (42% and 35% resp.).

IT **3211-76-5, Selenomethionine**  
RL: ANT (Analyte); ANST (Analytical study)  
(selenoamino acid speciation using HPLC-ETAAS following an enzymic hydrolysis of selenoprotein)

L15 ANSWER 11 OF 13 HCAPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 1995:757425 HCAPLUS  
DOCUMENT NUMBER: 123:136853  
TITLE: Selenophosphate synthetase: detection in extracts of rat tissues by immunoblot assay and partial purification of the enzyme from the archaean *Methanococcus vannielii*  
AUTHOR(S): Kim, Ick Young; Stadtman, Thressa C.  
CORPORATE SOURCE: Lab. Biochem., Natl. Inst. Health, Bethesda, MD, 20892-0320, USA  
SOURCE: Proceedings of the National Academy of Sciences of the United States of America (1995), 92(17), 7710-13  
CODEN: PNASA6; ISSN: 0027-8424  
PUBLISHER: National Academy of Sciences  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB In *Escherichia coli* and *Salmonella typhimurium* it has been shown that selenophosphate serves as the selenium donor for the conversion of seryl-tRNA to selenocysteyl-tRNA and for the synthesis of 2-selenouridine, a modified nucleoside present in tRNAs. Although selenocysteyl-tRNA also is formed in eukaryotes and is used for the specific insertion of **selenocysteine** into **proteins**, the precise mechanism of its biosynthesis from seryl-tRNA in these systems is not known. Because selenophosphate is extremely oxygen labile and difficult to identify in biol. systems, the authors used an immunol. approach to detect the possible presence of selenophosphate synthetase in mammalian tissues. With antibodies elicited to *E. coli* selenophosphate synthetase the enzyme was detected in **exts.** of rat brain, liver, kidney, and lung by immunoblotting. Especially high levels were detected in *Methanococcus vannielii*, a member of the domain Archaea, and the enzyme was partially purified from this source. It seems likely that the use of selenophosphate as a selenium donor is widespread in biol. systems.

L15 ANSWER 12 OF 13 HCAPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 1979:522166 HCAPLUS  
DOCUMENT NUMBER: 91:122166



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TITLE: Selenoamino acids  
INVENTOR(S): Sayuda, Kenji; Tanaka, Hidehiko  
PATENT ASSIGNEE(S): Ajinomoto Co., Inc., Japan  
SOURCE: Jpn. Kokai Tokkyo Koho, 6 pp.  
CODEN: JKXXAF  
DOCUMENT TYPE: Patent  
LANGUAGE: Japanese  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 54052033	A2	19790424	JP 1977-117664	19770929
JP 57008717	B4	19820217		

PRIORITY APPLN. INFO.: JP 1977-117664 19770929  
AB Eight selenoamino acids  $RSe(CH_2)_nCH(NH_2)CO_2H$  ( $R$  = organic residues;  $n = 1, 2$ ) were prepared by reaction of  $R_1(CH_2)_nCH(NH_2)CO_2H$  [ $R_1$  = halo,  $R_2O$  ( $R_2 = H, alkyl$ ),  $R_2S, R_2SO, R_2SO_2$ ] with  $RSeH$  in aqueous media in the presence of methioninase. Thus, *Pseudomonas ovalis* IFO 3738 was cultured on 1 kg of broth (pH 7.2) containing L-methionine 0.25, urea 0.1, peptone 0.1, glycerol 0.1,  $KH_2PO_4$  0.1,  $K_2HPO_4$  0.1,  $MgSO_4 \cdot 7H_2O$  0.01, and **yeast extract** 0.025 g/dL 18 h at  $28^\circ$  to give 2.2 kg cells, which were crushed in  $H_3PO_4$  buffer and the supernatant treated on DEAE-cellulose and Sephadex G-200 to give 280 mg enzyme **protein**. A mixture of 0.1M L-methionine (in 0.2M  $H_3PO_4$  buffer at pH 8.0), 0.1 mL 1M PhSeH (in EtOH), 0.5 mL 10-5M pyridoxal phosphate (in 0.02M  $H_3PO_4$  buffer at pH 8.0), and 1 mL of the enzyme liquid (50  $\mu g$  of **protein**/mL) was kept for 2 h at  $37^\circ$  under N with addition of 3 + 200  $\mu L$  of the enzyme liquid and 3 + 100  $\mu L$  the PhSeH liquid and the whole kept 25 min at  $100^\circ$  to give 4.3 mg  $\gamma$ -phenylseleno- $\alpha$ -aminobutyric acid [71128-79-5].

IT **3211-76-5P**  
RL: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)  
(manufacture of, by fermentation of *Pseudomonas ovalis*)

L15 ANSWER 13 OF 13 HCAPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 1977:66406 HCAPLUS  
DOCUMENT NUMBER: 86:66406  
TITLE: Assimilation, distribution, and metabolism of (75Se)-selenite, selenate, and selenoamino acids by *Escherichia coli*  
AUTHOR(S): Spallholz, Julian E.; Martin, John L.; Gerlach, Marlene L.  
CORPORATE SOURCE: Dep. Biochem., Colorado State Univ., Fort Collins, CO, USA  
SOURCE: Phosphorus and Sulfur and the Related Elements (1976), 1(2-3), 263-9  
CODEN: PREEDF; ISSN: 0308-664X  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB Assimilation of Se by *E. coli* as 75Se-labeled selenite, selenate, **selenomethionine** [1464-42-2], selenocystine [1464-43-3] and Se-CH<sub>3</sub>-selenocystine [2574-71-2] revealed that selenoamino acids from culture media were more competely assimilated than selenite or selenate and that the amount of selenite assimilated was three or four times that for selenate. Most (>95%) of the Se

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assimilated by *E. coli* was not solubilized by sonication and ethanol **extraction** but much (28% to 70%) of the Se, except Se from **selenomethionine**, was removed by alkaline dialysis. Se from selenocystine and from Se-CH<sub>3</sub>-selenocystine dialyzed from intact cells, whereas Se from selenite and selenate did not. Dialyzable Se is that Se probably present in selenotrisulfide (R-S-Se-R) bonds or bound nonspecifically. Anal. of the soluble Se metabolites from selenite, selenate, **selenomethionine** and selenocystine showed that *E. coli* produces at least one major metabolic product common to all substrates which upon chromatog. appeared to be selenocysteic acid [3614-09-3]. In monogastric animals selenite and selenate Se does not enter the primary **protein** structure as amino acids yet metabolites of selenite, selenate and selenocystine produced by *E. coli* may enter the primary **protein** structure of animals in min amts.

IT 1464-42-2

RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
(metabolism of, by *Escherichia coli*)

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO' ENTERED AT 15:29:38 ON 24 OCT 2003)

L16 19 S L15

L17 12 DUP REM L16 (7 DUPLICATES REMOVED)

L17 ANSWER 1 OF 12 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN

ACCESSION NUMBER: 2003:295442 SCISEARCH

THE GENUINE ARTICLE: 660HM

TITLE: Selenium speciation and isotope composition in Se-77-enriched yeast using gradient elution HPLC separation and ICP-dynamic reaction cell-MS  
AUTHOR: Larsen E H (Reprint); Sloth J; Hansen M; Moesgaard S  
CORPORATE SOURCE: Danish Vet & Food Adm, Inst Food Safety & Nutr, 19 Morkhoj Bygade, DK-2860 Soborg, Denmark (Reprint); Danish Vet & Food Adm, Inst Food Safety & Nutr, DK-2860 Soborg, Denmark; Pharma Nord Res Dept, DK-7100 Vejle, Denmark

COUNTRY OF AUTHOR: Denmark

SOURCE: JOURNAL OF ANALYTICAL ATOMIC SPECTROMETRY, (15 FEB 2003) Vol. 18, No. 4, pp. 310-316.  
Publisher: ROYAL SOC CHEMISTRY, THOMAS GRAHAM HOUSE, SCIENCE PARK, MILTON RD, CAMBRIDGE CB4 0WF, CAMBS, ENGLAND.

ISSN: 0267-9477.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 27

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB A batch of Se-77-labelled and enriched yeast was characterised with regard to isotopic composition and content of selenium species for later use in a human absorption study based on the method of enriched stable isotopes. The abundance of the six stable selenium isotopes was determined by ICP- MS equipped with a dynamic reaction cell ( DRC). The results showed that the Se-77 isotope was enriched to 98.5 atom-%, whereas the remaining selenium was present as the other five isotopes at low abundance. The low- molecular Se-77 containing species, which were biosynthesised by the yeast during fermentation using the enriched Se-77-selenite as substrate, were

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released by enzymatic hydrolysis using ( I), a beta- glucosidase followed by a protease mixture, and ( II), a commercial protease preparation. For selenium speciation the chromatographic selectivity of the cation exchange HPLC system was adjusted to the separation of over 30 selenium species occurring in the hydrolysates by applying gradient elution using pyridinium formate as mobile phase. The quantitative results obtained by detection with ICP-DRC-MS of Se-77 and Se-80 showed that both enzymatic sample preparation systems released 90 - 95% of the yeast's selenium content. The total area of the cation exchange chromatograms, however, amounted to 64% of the total selenium content in the **yeast**, which was 1390 mug g(-1). In the enzymatic **extracts selenomethionine** ( **SeMet**) constituted 82% of all separated and quantified selenium species, which was equivalent to 53% of the total selenium content in the yeast. Oxidation of **SeMet** to **selenomethionine- Se- oxide** ( **SeOMet**) occurred during sample preparation. The degree of formation of SeOMet was large and variable when using enzyme system I, but low when using enzyme II.

L17 ANSWER 2 OF 12 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN  
ACCESSION NUMBER: 2002-153866 [20] WPIDS  
DOC. NO. CPI: C2002-048028  
TITLE: A composition useful in the treatment of skin damages such as sunburn comprises reduced L-glutathione, selenium and epidermal growth factor, in a carrier.  
DERWENT CLASS: B04 B05 D16 D21  
INVENTOR(S): HERSH, T; WARSHAW, M A  
PATENT ASSIGNEE(S): (THIO-N) THIONE INT INC  
COUNTRY COUNT: 1  
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 6337320	B1	20020108	(200220)*		14

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
US 6337320	B1	US 1996-728948	19961011

PRIORITY APPLN. INFO: US 1996-728948 19961011

AN 2002-153866 [20] WPIDS

AB US 6337320 B UPAB: 20020402

NOVELTY - A composition comprises reduced L-glutathione, selenium and epidermal growth factor in a carrier.

ACTIVITY - Dermatological; Vulnerary.

No biological data given.

MECHANISM OF ACTION - Epidermal cell growth stimulator.

USE - In the treatment of skin damages caused by ultraviolet radiation (claimed) e.g. sunburn, photoaging and cutaneous malignancies.

ADVANTAGE - The composition reduces the effects of ultraviolet radiation induced skin damage and hence promotes skin repair and wound healing from a cute sunburn and chronic ultraviolet radiation induced photoaging processes in the skin.

Searcher : Shears 308-4994

Dwg.0/0

L17 ANSWER 3 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2002:422571 BIOSIS  
 DOCUMENT NUMBER: PREV200200422571  
 TITLE: Synthesis of **proteins** with disulfide bonds in *E. coli* using defined culture media.  
 AUTHOR(S): Fernandez-Tornero, C.; Ramon, A.; Navarro, M. L.; Varela, J.; Gimenez-Gallego, G. [Reprint author]  
 CORPORATE SOURCE: Centro de Investigaciones Biologicas, CSIC, Velazquez 144, Madrid, 28006, Spain  
 SOURCE: gimenez\_gallego@cib.csic.es  
 BioTechniques, (June, 2002) Vol. 32, No. 6, pp. 1238-1242. print.  
 CODEN: BTNQDO. ISSN: 0736-6205.  
 DOCUMENT TYPE: Article  
 LANGUAGE: English  
 ENTRY DATE: Entered STN: 7 Aug 2002  
 Last Updated on STN: 7 Aug 2002

L17 ANSWER 4 OF 12 MEDLINE on STN DUPLICATE 1  
 ACCESSION NUMBER: 2002311007 MEDLINE  
 DOCUMENT NUMBER: 22050820 PubMed ID: 12054777  
 TITLE: Crystal structure of the V-region of *Streptococcus mutans* antigen I/II at 2.4 A resolution suggests a sugar preformed binding site.  
 AUTHOR: Troffer-Charlier Nathalie; Ogier Joelle; Moras Dino; Cavarelli Jean  
 CORPORATE SOURCE: Institut National de la Sante et de la Recherche Medicale U424, Faculte de Chirurgie Dentaire, Universite Louis Pasteur, 11 rue Humann, 67085 Strasbourg, France.  
 SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (2002 Apr 19) 318 (1) 179-88.  
 Journal code: 2985088R. ISSN: 0022-2836.  
 PUB. COUNTRY: England: United Kingdom  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 OTHER SOURCE: PDB-1JMM  
 ENTRY MONTH: 200207  
 ENTRY DATE: Entered STN: 20020611  
 Last Updated on STN: 20020713  
 Entered Medline: 20020712

AB Antigens I/II are large multifunctional adhesins from oral viridans streptococci that exert immunomodulatory effects on human cells and play important roles in inflammatory disorders. Among them, *Streptococcus mutans* plays a major role in the initiation of dental caries. The structure of the V-region (SrV+, residues 464-840) of the antigen I/II of *S. mutans* has been determined using the multiwavelength anomalous diffraction phasing technique with **seleno-methionine**-substituted recombinant **protein** and subsequently refined at 2.4 A resolution. The crystal structure of SrV+ revealed a lectin-like fold that displays a putative preformed carbohydrate-binding site stabilized by a metal ion. Inhibition of this binding site may confer to humans a protection against dental caries and dissemination of the

**bacteria** to **extra**-oral sites involved in life-threatening inflammatory diseases. This crystal structure constitutes a first step in understanding the structure-function relationship of antigens I/II and may help in delineating new preventive or therapeutic strategies against colonization of the host by oral streptococci.

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L17 ANSWER 5 OF 12 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
 ACCESSION NUMBER: 2001:978905 SCISEARCH  
 THE GENUINE ARTICLE: 498MY  
 TITLE: Speciation of selenoamino acids, selenonium ions and inorganic selenium by ion exchange HPLC with mass spectrometric detection and its application to yeast and algae  
 AUTHOR: Larsen E H (Reprint); Hansen M; Fan T; Vahl M  
 CORPORATE SOURCE: Danish Vet & Food Adm, Inst Food Res & Nutr, 19 Morkhoj Bygade, DK-2860 Soborg, Denmark (Reprint); Danish Vet & Food Adm, Inst Food Res & Nutr, DK-2860 Soborg, Denmark; Univ Calif Davis, Dept Land Air & Water Resources, Davis, CA 95616 USA  
 COUNTRY OF AUTHOR: Denmark; USA  
 SOURCE: JOURNAL OF ANALYTICAL ATOMIC SPECTROMETRY, (NOV 2001 Vol. 16, No. 12, pp. 1403-1408. Publisher: ROYAL SOC CHEMISTRY, THOMAS GRAHAM HOUSE, SCIENCE PARK, MILTON RD,, CAMBRIDGE CB4 0WF, CAMBS, ENGLAND. ISSN: 0267-9477.  
 DOCUMENT TYPE: Article; Journal  
 LANGUAGE: English  
 REFERENCE COUNT: 31

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Cation and anion exchange HPLC were used to separate a mixture of 12 selenium species comprising selenoamino acids, selenonium ions and inorganic selenium. The cationic species were separated from each other and from the co-injected anions using a cation exchange column with gradient elution by aqueous pyridinium formate at pH similar to 3 as the mobile phase. The anionic species were separated using an anion exchange column with isocratic elution by an aqueous salicylate-TRIS mobile phase at pH 8.5. The separated selenium species were detected as Se-80 by ICP-dynamic reaction cell (DRC)-MS. The analytical methods were applied to the analysis of yeast and algae enriched in selenium. The yeast was treated with beta -glucosidase followed by a protease mixture for dissolution of the cell walls and selenium-containing **peptides**, respectively. The second to largest HPLC peak after that corresponding to **selenomethionine** was ascribed to **selenomethionine**-Se-oxide (SeOMet) by retention time matching with a SeOMet standard, which was characterised by HPLC-electrospray (ES)-MS. Se-methylselenocysteine was detected based on co-chromatography with the standard substance spiked to the **yeast** hydrolysate. A trichloroacetic acid **extract** of Chlorella algae contained dimethylselenonium propionate (DMSeP), which was verified by HPLC-ES-MS. Se-allylselenocysteine and selenoethionine was detected at the low ng g(-1) concentration level based on co-chromatography with the standard substances spiked to the algal extract.

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L17 ANSWER 6 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on  
STN DUPLICATE 2

ACCESSION NUMBER: 2001:329186 BIOSIS  
DOCUMENT NUMBER: PREV200100329186  
TITLE: The use of radiofrequency glow discharge-mass spectrometry (rf-GD-MS) coupled to gas chromatography for the determination of selenoaminoacids in biological samples.  
AUTHOR(S): Bayon, Maria Montes [Reprint author]; B'Hymer, Clayton [Reprint author]; Ponce de Leon, Claudia A. [Reprint author]; Caruso, Joseph A. [Reprint author]  
CORPORATE SOURCE: Department of Chemistry, University of Cincinnati, Cincinnati, OH, 45221-0172, USA  
joseph.caruso@uc.edu  
SOURCE: Journal of Analytical Atomic Spectrometry, (May, 2001) Vol. 16, No. 5, pp. 492-497. print.  
CODEN: JASPE2. ISSN: 0267-9477.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 11 Jul 2001  
Last Updated on STN: 19 Feb 2002

AB A radiofrequency glow discharge (rf-GD) has been used as an ion source for mass spectrometry using gas chromatography as the sample introduction technique for the speciation of seleno amino acids. The use of helium as the plasma gas allowed the monitoring of selenium's major isotope (80Se), interfered by polyatomic species when using Ar as the plasma gas. Optimization of the power and pressure of the source was undertaken to achieve the best sensitivity for selenium determination and detection limits of about 100 pg (as Se) have been obtained for the derivatized **Se-methionine**. Studying the cell pressure and power on the fragmentation patterns obtained for **Se-methionine** and Se-ethionine has also been undertaken to characterize the source. No significant variations were observed on the mass spectra different operation conditions, although noticeable variation of the fragment intensities was observed. The system has been applied to the determination of **Se-methionine** in selenium-enriched **yeast** after enzymatic **extraction** of the corresponding species using Proteinase K to cleave the **peptide** bonds. The results obtained showed a **Se-methionine** content of about 78% in the enriched yeast.

L17 ANSWER 7 OF 12 MEDLINE on STN DUPLICATE 3  
ACCESSION NUMBER: 95372349 MEDLINE  
DOCUMENT NUMBER: 95372349 PubMed ID: 7644481  
TITLE: Selenophosphate synthetase: detection in extracts of rat tissues by immunoblot assay and partial purification of the enzyme from the archaean Methanococcus vannielii.  
AUTHOR: Kim I Y; Stadtman T C  
CORPORATE SOURCE: Laboratory of Biochemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892-0320, USA.  
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1995 Aug 15) 92 (17) 7710-3.  
Journal code: 7505876. ISSN: 0027-8424.  
PUB. COUNTRY: United States

Searcher : Shears 308-4994

09/989974

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199509  
ENTRY DATE: Entered STN: 19950930  
Last Updated on STN: 19990129  
Entered Medline: 19950921

AB In *Escherichia coli* and *Salmonella typhimurium* it has been shown that selenophosphate serves as the selenium donor for the conversion of seryl-tRNA to selenocysteyl-tRNA and for the synthesis of 2-selenouridine, a modified nucleoside present in tRNAs. Although selenocysteyl-tRNA also is formed in eukaryotes and is used for the specific insertion of **selenocysteine** into **proteins**, the precise mechanism of its biosynthesis from seryl-tRNA in these systems is not known. Because selenophosphate is extremely oxygen labile and difficult to identify in biological systems, we used an immunological approach to detect the possible presence of selenophosphate synthetase in mammalian tissues. With antibodies elicited to *E. coli* selenophosphate synthetase the enzyme was detected in **extracts** of rat brain, liver, kidney, and lung by immunoblotting. Especially high levels were detected in *Methanococcus vannielii*, a member of the domain Archaea, and the enzyme was partially purified from this source. It seems likely that the use of selenophosphate as a selenium donor is widespread in biological systems.

L17 ANSWER 8 OF 12 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 96033547 EMBASE  
DOCUMENT NUMBER: 1996033547  
TITLE: Assessment of selenium chemical species in selenium-yeast.  
AUTHOR: Yang L.; Hou S.; Li D.  
CORPORATE SOURCE: Institute of Geography, Chinese Academy of Sciences, Beijing 100101, China  
SOURCE: Chinese Pharmaceutical Journal, (1995) 30/11 (680-682).  
ISSN: 1001-2494 CODEN: ZYZAEU  
COUNTRY: China  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: Chinese  
SUMMARY LANGUAGE: Chinese; English

AB Selenium chemical species in selenium-**yeast** were determined by **extraction** with tris-buffer-glycine mixed solution, and compared with dialysis method. The results indicated that more than 50% of selenium in the selenium-yeast (total Se is 4000 µg/g) was probably in **protein** bound molecules as **selenomethionine** or other large organic species. The inorganic species only accounted for 3%, and the free species 0.01%. It was shown that Se yeast has development prospectives and there is a better way for determination of selenium products.

L17 ANSWER 9 OF 12 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
ACCESSION NUMBER: 95:828690 SCISEARCH  
THE GENUINE ARTICLE: TG189  
TITLE: SELENOAMINO ACID SPECIATION USING HPLC-ETAAS FOLLOWING AN ENZYMATIC-HYDROLYSIS OF SELENOPROTEIN

Searcher : Shears 308-4994

AUTHOR: GILON N; ASTRUC A; ASTRUC M; POTINGAUTIER M  
(Reprint)  
CORPORATE SOURCE: UNIV PAU & PAYS ADOUR, CURS, CHIM ANALYT LAB, AVE  
UNIV, F-64000 PAU, FRANCE (Reprint); UNIV PAU & PAYS  
ADOUR, CURS, CHIM ANALYT LAB, F-64000 PAU, FRANCE  
COUNTRY OF AUTHOR: FRANCE  
SOURCE: APPLIED ORGANOMETALLIC CHEMISTRY, (NOV 1995) Vol. 9,  
No. 7, pp. 623-628.  
ISSN: 0268-2605.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: PHYS  
LANGUAGE: ENGLISH  
REFERENCE COUNT: 24

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB A high-pressure liquid chromatography-electrothermal atomic  
absorption spectroscopy (HPLC-ETAAS) hyphenated technique was used  
for the determination of seleno compounds present in a  
selenium-enriched yeast. Conditions were optimized for the  
separation and quantification of the selenoamino acids,  
selenocystine and **selenomethionine**, in the presence of  
other compounds. The separation was achieved by ion-pairing  
chromatography using sodium heptanesulphonate as the anionic  
counterion. On-line detection was carried out using electrothermal  
atomic absorption with palladium(II) as a matrix modifier. Different  
**extraction** procedures were tested on a selenium-enriched  
**yeast**. A 92% recovery of the total selenium present in the  
material was obtained. Attempts to evaluate selenium speciation were  
carried out; **selenomethionine** and selenocystine were  
identified as the major components (42% and 35% respectively).

L17 ANSWER 10 OF 12 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
ACCESSION NUMBER: 95:814361 SCISEARCH  
THE GENUINE ARTICLE: TF485  
TITLE: TRANSCRIPTION OF ESCHERICHIA-COLI tRNA(SEC) GENE BY  
RNA-POLYMERASE-II IN SACCHAROMYCES-CEREVISIAE  
AUTHOR: ZHANG M H (Reprint); LIU J H; JIN Y X  
CORPORATE SOURCE: CHINESE ACAD SCI, SHANGHAI INST BIOCHEM, STATE KEY  
LAB MOLEC BIOL, SHANGHAI 200031, PEOPLES R CHINA  
COUNTRY OF AUTHOR: PEOPLES REPUBLIC OF CHINA  
SOURCE: ACTA BIOCHIMICA ET BIOPHYSICA SINICA, (SEP 1995)  
Vol. 27, No. 5, pp. 529-536.  
ISSN: 0582-9879.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: Chinese  
REFERENCE COUNT: 14

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The **selenocysteine** tRNA (SelC) gene of E. coli was  
inserted into a secretory expression plasmid vector, PVT102U-alpha  
MFL, with adjusted reading frame of **protein** translation,  
and added stop codon at the downstream of the structure gene. Its  
expression was established in Saccharomyces cerevisiae. A 7-8kd band  
in SD culture media was shown by SDS-PAGE analysis to be consistent  
with the theoretical value. The total RNA was **extracted**  
from **yeast** and Northern blot was done with it 5'  
end-labeled 21-mer oligonucleotide probe complementary to the T psi  
C region of the tRNA(Sec). The result showed that beside one weak  
band there were two strong bands, one of which corresponded to 790  
nts and the other, 370 nts. It is suggested that the 790 nts band is



the unprocessed precursor of RNA polymerase II-mediated transcript, the 370 nts band is RNA molecules processed at the 5'-ends but not the 3'-ends, and the weaker band is the mature tRNA of about 90nts long. We concluded that RNA polIII could transcribe tRNA genes. Because of the low level of 3'-endonuclease in the yeast strain we used, the 3'-end processing of the precursors with long tails was slow.

L17 ANSWER 11 OF 12 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
 ACCESSION NUMBER: 92:345139 SCISEARCH  
 THE GENUINE ARTICLE: HW764  
 TITLE: PURIFICATION AND CHARACTERIZATION OF **PROTEIN**  
 -P(C), A COMPONENT OF GLYCINE REDUCTASE FROM  
 EUBACTERIUM-ACIDAMINOPHILUM  
 AUTHOR: SCHRADER T; ANDREESSEN J R (Reprint)  
 CORPORATE SOURCE: UNIV GOTTINGEN, INST MIKROBIOL, GRISEBACHSTR 8,  
 W-3400 GOTTINGEN, GERMANY  
 COUNTRY OF AUTHOR: GERMANY  
 SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, (15 MAY 1992) Vol.  
 206, No. 1, pp. 79-85.  
 ISSN: 0014-2956.  
 DOCUMENT TYPE: Article; Journal  
 FILE SEGMENT: LIFE  
 LANGUAGE: ENGLISH  
 REFERENCE COUNT: 36

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB **Protein** P(C) of the glycine reductase from Eubacterium acidaminophilum was purified to homogeneity by chromatography on phenyl-Sepharose and Sepharose S. The apparent molecular mass of the native **protein**, which showed an associating/dissociating behaviour, was about 420 kDa. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis of **protein** P(C) revealed two **protein** bands corresponding to 48 and 57 kDa, indicating an alpha-4-beta-4 composition. The smaller subunit was identified as an acetyl-group-transferring **protein**, the 57-kDa **protein** was hydrophobic. N-terminal amino acid sequences were determined for both subunits. Antibodies raised against the 48-kDa subunit showed cross-reactions with extracts of E. acidaminophilum grown on different substrates and with **extracts** from other glycine-utilizing anaerobic **bacteria** such as Clostridium purinolyticum, C. sticklandii, and C. sporogenes. The respective **protein** from the former two organisms corresponded in molecular mass. When **protein** P(A) was chemically carboxymethylated by iodo[2-C-14]acetate and incubated with **protein** P(C), acetyl phosphate was a reaction product, thus establishing it as the product of the glycine reductase reaction by using homogeneous preparations of these two **proteins** from E. acidaminophilum.

L17 ANSWER 12 OF 12 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS  
 RESERVED. on STN  
 ACCESSION NUMBER: 90190749 EMBASE  
 DOCUMENT NUMBER: 1990190749  
 TITLE: Purification and biochemical characterization of  
 SELB, a translation factor involved in selenoprotein  
 synthesis.  
 AUTHOR: Forchhammer K.; Rucknagel K.-P.; Bock A.  
 CORPORATE SOURCE: Lehrstuhl fur Mikrobiologie, Universitat Munchen,

09/989974

SOURCE: Maria-Ward-Strasse 1a, D-8000 Munchen 19, Germany  
Journal of Biological Chemistry, (1990) 265/16  
(9346-9350).  
ISSN: 0021-9258 CODEN: JBCHA3  
COUNTRY: United States  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: English  
SUMMARY LANGUAGE: English

AB The product of the selB gene from Escherichia coli is required for co-translational insertion of **selenocysteine** into **protein**. To make the SELB **protein** accessible to biochemical analysis, the **protein** was purified from cells that overexpressed the selB gene from a phage T7 promoter plasmid. It was calculated that the overproduced SELB **protein** was purified 20-fold. The N-terminal amino acid sequence of the purified **protein** was determined, and it confirmed that the initiation codon of selB mRNA translation overlaps the stop codon of the preceding selA gene by 4 bases. Structural similarity between SELB and elongation factors was demonstrated by limited proteolysis of SELB by trypsin. The cleavage sites within SELB were identified by N-terminal sequencing of the two proteolytic products. The position in the SELB **protein** of the major cleavage site was homologous to a tryptic cleavage site which is characteristic for elongation factors. Immunological analysis showed that the levels of SELB are equivalent in aerobically and anaerobically grown cells; the amount of the **protein** was estimated to be approximately 1100 copies/E. coli cell. Upon fractionation of cell **extracts**, SELB was found to be partially associated with the ribosomes. The results therefore indicate that SELB is the first known elongation factor-like **protein** that has specificity for a particular charged tRNA.

(FILE 'MEDLINE' ENTERED AT 15:30:38 ON 24 OCT 2003)

L18 394 SEA FILE=MEDLINE ABB=ON PLU=ON SELENOCYSTEINE/CT  
L19 751 SEA FILE=MEDLINE ABB=ON PLU=ON SELENOMETHIONINE/CT  
L20 120010 SEA FILE=MEDLINE ABB=ON PLU=ON PROTEINS/CT  
L21 154 SEA FILE=MEDLINE ABB=ON PLU=ON L20 AND (L18 OR L19)  
L22 155049 SEA FILE=MEDLINE ABB=ON PLU=ON "ESCHERICHIA COLI"/CT  
L23 8788 SEA FILE=MEDLINE ABB=ON PLU=ON YEASTS/CT  
L24 55770 SEA FILE=MEDLINE ABB=ON PLU=ON BACTERIA/CT  
L25 23 SEA FILE=MEDLINE ABB=ON PLU=ON L21 AND (L23 OR L22 OR L24)

L25 ANSWER 1 OF 23 MEDLINE on STN

AN 2003308334 MEDLINE

TI Selenomethionine incorporation into a protein by cell-free synthesis.

AU Kigawa Takanori; Yamaguchi-Nunokawa Emi; Kodama Koichiro; Matsuda Takayoshi; Yabuki Takashi; Matsuda Natsuko; Ishitani Ryuichiro; Nureki Osamu; Yokoyama Shigeyuki

SO J Struct Funct Genomics, (2002) 2 (1) 29-35.  
Journal code: 101128185. ISSN: 1345-711X.

AB Multi-wavelength anomalous diffraction phasing is especially useful for high-throughput structure determinations. Selenomethionine substituted proteins are commonly used for this purpose. However, the cytotoxicity of selenomethionine drastically reduces the efficiency of its incorporation in in vivo expression systems. In

the present study, an improved *E. coli* cell-free protein synthesis system was used to incorporate selenomethionine into a protein, so that highly efficient incorporation could be achieved. A milligram quantity of selenomethionine-containing Ras was obtained using the cell-free system with dialysis. The mass spectrometry analysis showed that more than 95% of the methionine residues were substituted with selenomethionine. The crystal of this protein grew under the same conditions and had the same unit cell constants as those of the native Ras protein. The three-dimensional structure of this protein, determined by multi-wavelength anomalous diffraction phasing, was almost the same as that of the Ras protein prepared by *in vivo* expression. Therefore, the cell-free synthesis system could become a powerful protein expression method for high-throughput structure determinations by X-ray crystallography.

- L25 ANSWER 2 OF 23 MEDLINE on STN  
 AN 2002167187 MEDLINE  
 TI Selenoprotein biosynthesis: purification and assay of components involved in selenocysteine biosynthesis and insertion in *Escherichia coli*.  
 AU Thanbichler Martin; Bock August  
 SO METHODS IN ENZYMOLOGY, (2002) 347 3-16.  
 Journal code: 0212271. ISSN: 0076-6879.
- L25 ANSWER 3 OF 23 MEDLINE on STN  
 AN 2002167180 MEDLINE  
 TI Transfer RNAs that insert selenocysteine.  
 AU Carlson Bradley A; Hatfield Dolph L  
 SO METHODS IN ENZYMOLOGY, (2002) 347 24-39.  
 Journal code: 0212271. ISSN: 0076-6879.
- L25 ANSWER 4 OF 23 MEDLINE on STN  
 AN 2002167178 MEDLINE  
 TI Recombinant expression of mammalian selenocysteine-containing thioredoxin reductase and other selenoproteins in *Escherichia coli*.  
 AU Arner Elias S J  
 SO METHODS IN ENZYMOLOGY, (2002) 347 226-35. Ref: 48  
 Journal code: 0212271. ISSN: 0076-6879.
- L25 ANSWER 5 OF 23 MEDLINE on STN  
 AN 2001532935 MEDLINE  
 TI Utilization of selenocysteine as a source of selenium for selenophosphate biosynthesis.  
 AU Lacourciere G M; Stadtman T C  
 SO BIOFACTORS, (2001) 14 (1-4) 69-74. Ref: 22  
 Journal code: 8807441. ISSN: 0951-6433.
- AB Selenophosphate synthetase (SPS), the *seld* gene product from *Escherichia coli*, catalyzes the biosynthesis of monoselenophosphate from selenide and ATP. Characterization of selenophosphate synthetase revealed the determined  $K(m)$  value for selenide is far above the optimal concentration needed for growth and approached levels which are toxic. Selenocysteine lyase enzymes, which decompose selenocysteine to elemental selenium (Se(0)) and alanine, were considered as candidates for the control of free selenium levels *in vivo*. The ability of a lyase protein to generate Se(0) in the proximity of SPS maybe an attractive solution to selenium toxicity as well as the high  $K(m)$  value for selenide. Recently, three *E. coli* NifS-like proteins, CsdB, CSD, and IscS, were

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characterized. All three proteins exhibit lyase activity on L-cysteine and L-selenocysteine and produce sulfane sulfur, S(0), or Se(0) respectively. Each lyase can effectively mobilize Se(0) from L-selenocysteine for selenophosphate biosynthesis.

- L25 ANSWER 6 OF 23 MEDLINE on STN  
AN 2001521759 MEDLINE  
TI Evolution of selenocysteine-containing proteins: significance of identification and functional characterization of selenoproteins.  
AU Gladyshev V N; Kryukov G V  
SO BIOFACTORS, (2001) 14 (1-4) 87-92. Ref: 30  
Journal code: 8807441. ISSN: 0951-6433.  
AB In the genetic code, UGA serves as either a signal for termination or a codon for selenocysteine (Sec). Sec rarely occurs in protein and is different from other amino acids in that much of the biosynthetic machinery governing its incorporation into protein is unique to this amino acid. Sec-containing proteins have diverse functions and lack a common amino acid motif or consensus sequence. Sec has previously been considered to be a relic of the primordial genetic code that was counter-selected by the presence of oxygen in the atmosphere. In the present report, it is proposed that Sec was added to the already existing genetic code and its use has accumulated during evolution of eukaryotes culminating in vertebrates. The more recently evolved selenoproteins appear to take advantage of unique redox properties of Sec that are superior to those of Cys for specific biological functions. Further understanding of the evolution of selenoproteins as well as biological properties and biomedical applications of the trace element selenium requires identification and functional characterization of all mammalian selenoproteins.
- L25 ANSWER 7 OF 23 MEDLINE on STN  
AN 2000116055 MEDLINE  
TI UGA: a dual signal for 'stop' and for recoding in protein synthesis.  
AU Tate W P; Mansell J B; Mannering S A; Irvine J H; Major L L; Wilson D N  
SO BIOCHEMISTRY, (1999 Dec) 64 (12) 1342-53. Ref: 48  
Journal code: 0376536. ISSN: 0006-2979.  
AB UGA remains an enigma as a signal in protein synthesis. Long recognized as a stop signal that is prone to failure when under competition from near cognate events, there was growing belief that there might be functional significance in the production of small amounts of extended proteins. This view has been reinforced with the discovery that UGA is found at some recoding sites where frameshifting occurs as a regulatory mechanism for controlling the gene expression of specific proteins, and it also serves as the code for selenocysteine (Sec), the 21st amino acid. Why does UGA among the stop signals play this role specifically, and how does it escape being used to stop protein synthesis efficiently at recoding sites involving Sec incorporation or shifts to a new translational frame? These issues concerning the UGA stop signals are discussed in this review.
- L25 ANSWER 8 OF 23 MEDLINE on STN  
AN 2000105454 MEDLINE  
TI The efficiency of Escherichia coli selenocysteine insertion is influenced by the immediate downstream nucleotide.  
AU Sandman K E; Noren C J

- SO NUCLEIC ACIDS RESEARCH, (2000 Feb 1) 28 (3) 755-61.  
Journal code: 0411011. ISSN: 1362-4962.
- AB Selenocysteine (Sec) incorporation requires the TGA opal codon and a downstream Sec insertion sequence (SECIS), which can be partially randomized and cloned into M13 pIII fusion constructs for phage display. This combinatorial approach provides a convenient non-radioactive assay that couples phage production to opal suppression. Two SECIS libraries were prepared, with the immediate downstream nucleotide either randomized (TGAN) or fixed as thymidine (TGAT). The TGAN library resulted in a majority of clones with a downstream purine and selenium-independent phage production, implicating the endo-genous tryptophan-inserting opal suppression pathway. Although the addition of sodium selenite to the growth medium did not affect phage production, it did increase the level of Sec insertion, as shown by the chemical reactivity of the resulting phage. The TGAT phage library yielded clones with strictly selenium-dependent phage production and reactivity consistent with the presence of Sec. These clones were prone to spontaneous mutation upon further propagation, however, resulting in loss of the selenium-dependent phenotype. We conclude that the immediate downstream nucleotide determines whether the endogenous opal suppression pathway competes with co-translational Sec insertion.
- L25 ANSWER 9 OF 23 MEDLINE on STN  
AN 2000003159 MEDLINE  
TI Crystallization and preliminary x-ray diffraction studies on the conserved GTPase domain of the signal recognition particle from *Acidianus ambivalens*.  
AU Montoya G; te Kaat K; Moll R; Schafer G; Sinning I  
SO ACTA CRYSTALLOGRAPHICA. SECTION D: BIOLOGICAL CRYSTALLOGRAPHY, (1999 Nov) 55 (11) 1949-51.  
Journal code: 9305878. ISSN: 0907-4449.
- AB The signal recognition particle (SRP) of bacteria consists of only one protein, known as Ffh or the SRP54 homologue, which forms a complex with 4.5S RNA. It also binds to signal peptides and contains a GTPase which displays interesting differences to Ras GTPases. The conserved NG-domain of Ffh from the archaebacterium *Acidianus ambivalens* was cloned and overexpressed with a C-terminal His tag in *Escherichia coli*. Crystallization experiments of the native protein as well as of the Thr112Ala mutant, which is deficient in GTP hydrolysis, resulted in crystals suitable for X-ray diffraction. The crystals belong to the orthorhombic space group C222(1), with unit-cell parameters  $a = 64.5$ ,  $b = 128.3$ ,  $c = 72.0$  Å. At cryogenic temperatures, the crystals diffracted to a resolution limit of 2.8 Å using a rotating-anode generator and contain one molecule per asymmetric unit. A native data set has been collected using synchrotron radiation to around 2.0 Å resolution. Selenomethionine protein was produced; its crystals diffract in-house to about 2.8 Å resolution.
- L25 ANSWER 10 OF 23 MEDLINE on STN  
AN 2000003156 MEDLINE  
TI Expression of a selenomethionyl derivative and preliminary crystallographic studies of human cystatin C.  
AU Kozak M; Jankowska E; Janowski R; Grzonka Z; Grubb A; Alvarez Fernandez M; Abrahamson M; Jaskolski M  
SO ACTA CRYSTALLOGRAPHICA. SECTION D: BIOLOGICAL CRYSTALLOGRAPHY, (1999 Nov) 55 (11) 1939-42.

- Journal code: 9305878. ISSN: 0907-4449.
- AB Human cystatin C, a protein with amyloidogenic properties and a potent inhibitor of papain-like mammalian proteases, has been produced in its full-length form by recombinant techniques and crystallized in two polymorphic forms: cubic and tetragonal. A selenomethionyl derivative of the protein, obtained by *Escherichia coli* expression and with complete Met-->Se-Met substitution confirmed by mass spectrometry, amino-acid analysis and X-ray absorption spectra, was crystallized in the cubic form. A truncated variant of the protein, lacking ten N-terminal residues, has also been crystallized. The crystals of this variant are tetragonal and, like the two polymorphs of the full-length protein, contain multiple copies of the molecule in the asymmetric unit, suggesting oligomerization of the protein.
- L25 ANSWER 11 OF 23 MEDLINE on STN
- AN 1999443884 MEDLINE
- TI High-level expression in *Escherichia coli* of selenocysteine-containing rat thioredoxin reductase utilizing gene fusions with engineered bacterial-type SECIS elements and co-expression with the *selA*, *selB* and *selC* genes.
- AU Arner E S; Sarioglu H; Lottspeich F; Holmgren A; Bock A
- SO JOURNAL OF MOLECULAR BIOLOGY, (1999 Oct 8) 292 (5) 1003-16.  
Journal code: 2985088R. ISSN: 0022-2836.
- AB Mammalian thioredoxin reductase (TrxR) catalyzes reduction of thioredoxin and many other substrates, and is a central enzyme for cell proliferation and thiol redox control. The enzyme is a selenoprotein and can therefore, like all other mammalian selenoproteins, not be directly expressed in *Escherichia coli*, since selenocysteine-containing proteins are synthesized by a highly species-specific translation machinery. This machinery involves a secondary structure, SECIS element, in the selenoprotein-encoding mRNA, directing selenocysteine insertion at the position of an opal (UGA) codon, normally conferring termination of translation. It is species-specific structural features and positions in the selenoprotein mRNA of the SECIS elements that hitherto have hampered heterologous production of recombinant selenoproteins. We have discovered, however, that rat TrxR can be expressed in *E. coli* by fusing its open reading frame with the SECIS element of the bacterial selenoprotein formate dehydrogenase H. A variant of the SECIS element designed to encode the conserved carboxyterminal end of the enzyme (-Sec-Gly-COOH) and positioning parts of the SECIS element in the 3'-untranslated region was also functional. This finding revealed that the SECIS element in bacteria does not need to be translated for full function and it enabled expression of enzymatically active mammalian TrxR. The recombinant selenocysteine-containing TrxR was produced at dramatically higher levels than formate dehydrogenase O, the only endogenous selenoprotein expressed in *E. coli* under the conditions utilized, demonstrating a surprisingly high reserve capacity of the bacterial selenoprotein synthesis machinery under aerobic conditions. Co-expression with the *selA*, *selB* and *selC* genes (encoding selenocysteine synthase, SELB and tRNA(Sec), respectively) further increased the efficiency of the selenoprotein production and thereby also increased the specific activity of the recombinant TrxR to about 25 % of the native enzyme, with as much as 20 mg produced per liter of culture. These results show that with the strategy utilized here, the capacity of selenoprotein synthesis in *E. coli* is

more than sufficient for making possible the use of the bacteria for production of recombinant selenoproteins.  
Copyright 1999 Academic Press.

- L25 ANSWER 12 OF 23 MEDLINE on STN  
AN 1998060719 MEDLINE  
TI Functional expression in *Escherichia coli* of the *Haemophilus influenzae* gene coding for selenocysteine-containing selenophosphate synthetase.  
AU Wilting R; Vamvakidou K; Bock A  
SO ARCHIVES OF MICROBIOLOGY, (1998 Jan) 169 (1) 71-5.  
Journal code: 0410427. ISSN: 0302-8933.  
AB The selenophosphate synthetases from several organisms contain a selenocysteine residue in their active site where the *Escherichia coli* enzyme contains a cysteine. The synthesis of these enzymes, therefore, depends on their own reaction product. To analyse how this self-dependence is correlated with the selenium status, e.g. after recovery from severe selenium starvation, we expressed the gene for the selenocysteine-containing selenophosphate synthetase from *Haemophilus influenzae* (selDHI) in an *E. coli* DeltaselD strain. Gene selDHI gave rise to a selenium-containing gene product and also supported - via its activity - the formation of *E. coli* selenoproteins. The results provide evidence either for the suppression of the UGASec codon with the insertion of an amino acid allowing the formation of a functional product or for a bypass of the selenophosphate requirement. We also show that the selenocysteine synthesis and the insertion systems of the two organisms are fully compatible despite conspicuous differences in the mRNA recognition motif.
- L25 ANSWER 13 OF 23 MEDLINE on STN  
AN 1998004568 MEDLINE  
TI The path of unspecific incorporation of selenium in *Escherichia coli*.  
AU Muller S; Heider J; Bock A  
SO ARCHIVES OF MICROBIOLOGY, (1997 Nov) 168 (5) 421-7.  
Journal code: 0410427. ISSN: 0302-8933.  
AB The path of unspecific selenium incorporation into proteins was studied in *Escherichia coli* mutants blocked in the biosynthesis of cysteine and methionine or altered in its regulation. Selenium incorporation required all enzymatic steps of cysteine biosynthesis except sulfite reduction, indicating that intracellular reduction of selenite occurs nonenzymatically. Cysteine (but not methionine) supplementation prevented unspecific incorporation of selenium by repressing cysteine biosynthesis. On the other hand, when the biosynthesis of cysteine was derepressed in regulatory mutants, selenium was incorporated to high levels. These findings and the fact that methionine auxotrophic strains still displayed unspecific incorporation show that selenium incorporation into proteins in *E. coli* occurs mainly as selenocysteine. These findings also provide information on the labeling conditions for incorporating <sup>75</sup>Se only and specifically into selenoproteins.
- L25 ANSWER 14 OF 23 MEDLINE on STN  
AN 97114083 MEDLINE  
TI RNAs mediating cotranslational insertion of selenocysteine in eukaryotic selenoproteins.  
AU Hubert N; Walczak R; Sturchler C; Myslinski E; Schuster C; Westhof

- E; Carbon P; Krol A  
 SO BIOCHIMIE, (1996) 78 (7) 590-6. Ref: 40  
 Journal code: 1264604. ISSN: 0300-9084.
- AB Selenocysteine, a selenium-containing analog of cysteine, is found in the prokaryotic and eukaryotic kingdoms in active sites of enzymes involved in oxidation-reduction reactions. Its biosynthesis and cotranslational insertion into selenoproteins is performed by an outstanding mechanism, implying the participation of several gene products. The tRNA(Sec) is one of these. In eukaryotes, its transcription mode by RNA polymerase III differs from that of classical tRNA genes, both at the level of the promoter elements and transcription factors involved. In addition, enhanced transcription is afforded by a newly characterized zinc finger activator. Not only transcription of the gene, but also the tRNA(Sec) itself is atypical since its 2D and 3D structures exhibit features which set it apart from classical tRNAs. Decoding of eukaryotic selenocysteine UGA codons requires a stem-loop structure in the 3'UTR of mRNAs, the selenocysteine insertion sequence (SECIS) element. Structure probing and sequence comparisons led us to propose a 2D structure model for the SECIS element, containing a novel RNA motif composed of four consecutive non-Watson-Crick base-pairs. A 3D model, rationalizing the accessibility data, was elaborated by computer modeling. It yields indicative or suggestive evidence for the role that could play some conserved residues and/or structural features in SECIS function. These might act as signals for interaction with SBP, the SECIS binding protein that we have characterized.
- L25 ANSWER 15 OF 23 MEDLINE on STN  
 AN 94310048 MEDLINE  
 TI Eukaryotic selenocysteine inserting tRNA species support selenoprotein synthesis in Escherichia coli.  
 AU Baron C; Sturchler C; Wu X Q; Gross H J; Krol A; Bock A  
 SO NUCLEIC ACIDS RESEARCH, (1994 Jun 25) 22 (12) 2228-33.  
 Journal code: 0411011. ISSN: 0305-1048.
- AB Although the tRNA species directing selenocysteine insertion in prokaryotes differ greatly in their primary structure from that of their eukaryotic homologues they share very similar three-dimensional structures. To analyse whether this conservation of the overall shape of the molecules reflects a conservation of their functional interactions it was tested whether the selenocysteine inserting tRNA species from Homo sapiens supports selenoprotein synthesis in E. coli. It was found that the expression of the human tRNA(Sec) gene in E.coli can complement a lesion in the tRNA(Sec) gene of this organism. Transcripts of the Homo sapiens and Xenopus laevis tRNA(Sec) genes synthesised in vitro were amino-acylated by the E.coli seryl-tRNA ligase although at a very low rate and the resulting seryl-tRNA(Sec) was bound to and converted into selenocysteyl-tRNA(Sec) by the selenocysteine synthase of this organism. Selenocysteyl-tRNA(Sec) from both eukaryotes was able to form a complex with translation factor SELB from E.coli. Although the mechanism of selenocysteine incorporation into seleno-proteins appears to be rather different in E.coli and in vertebrates, we observe here a surprising conservation of functions over an enormous evolutionary distance.

L25 ANSWER 16 OF 23 MEDLINE on STN  
 AN 94148228 MEDLINE



- TI Recognition of the mRNA selenocysteine insertion sequence by the specialized translational elongation factor SELB.
- AU Ringquist S; Schneider D; Gibson T; Baron C; Bock A; Gold L
- SO GENES AND DEVELOPMENT, (1994 Feb 1) 8 (3) 376-85.  
Journal code: 8711660. ISSN: 0890-9369.
- AB In *Escherichia coli* the unusual amino acid selenocysteine is incorporated cotranslationally at an in-frame UGA codon. Incorporation of selenocysteine relies, in part, on the interaction between a specialized elongation factor, the SELB protein, and a cis-acting element within the mRNA. Boundary and toeprint experiments illustrate that the SELB-GTP-Sec-tRNA(Sec) ternary complex binds to the selenoprotein encoding mRNAs *fdhF* and *fdnG*, serving to increase the concentration of SELB and Sec-tRNA(Sec) on these mRNAs in vivo. Moreover, toeprint experiments indicate that SELB recognizes the ribosome-bound message and that, upon binding, SELB may protrude out of the ribosomal-mRNA track so as to approach the large ribosomal subunit. The results place the mRNA-bound SELB-GTP-Sec-tRNA(Sec) ternary complex at the selenocysteine codon (as expected) and suggest a mechanism to explain the specificity of selenocysteine insertion. Cis-acting mRNA regulatory elements can tether protein factors to the translation complex during protein synthesis.
- L25 ANSWER 17 OF 23 MEDLINE on STN
- AN 92142222 MEDLINE
- TI Selenoprotein synthesis: an expansion of the genetic code.
- AU Bock A; Forchhammer K; Heider J; Baron C
- SO TRENDS IN BIOCHEMICAL SCIENCES, (1991 Dec) 16 (12) 463-7. Ref: 37  
Journal code: 7610674. ISSN: 0968-0004.
- AB A number of enzymes employ the unusual amino acid selenocysteine as part of their active site because of its high chemical reactivity. Selenocysteine is incorporated into these proteins co-translationally: biosynthesis occurs on a specific tRNA and insertion into a growing polypeptide is directed by a UGA codon in the mRNA. In *E. coli*, this requires a specific translation factor. Selenocysteine thus represents a unique expansion of the genetic code.
- L25 ANSWER 18 OF 23 MEDLINE on STN
- AN 92100196 MEDLINE
- TI [Biology and biochemistry of selenium].  
Biologie und Biochemie des Elements Selen.
- AU Forchhammer K; Bock A
- SO NATURWISSENSCHAFTEN, (1991 Nov) 78 (11) 497-504. Ref: 42  
Journal code: 0400767. ISSN: 0028-1042.
- AB The importance of selenium as an essential trace element has progressively emerged during the last years due to the analysis of selenium deficiency diseases and to the identification and characterization of a number of selenoenzymes. Selenium is incorporated in the catalytic site of the enzymes as an integral selenocysteine residue. The pathway of selenocysteine biosynthesis and incorporation has been elucidated recently for *Escherichia coli*. This article presents an overview on these subjects and describes the mechanisms which confer selenocysteine specificity in the framework of protein biosynthesis. In addition, some considerations concerning the phylogeny of selenocysteine incorporation are presented and a model for the evolution of the selenocysteine pathway is proposed.

- L25 ANSWER 19 OF 23 MEDLINE on STN  
 AN 90328737 MEDLINE  
 TI Selenium biochemistry.  
 AU Stadtman T C  
 SO ANNUAL REVIEW OF BIOCHEMISTRY, (1990) 59 111-27. Ref: 97  
 Journal code: 2985150R. ISSN: 0066-4154.
- L25 ANSWER 20 OF 23 MEDLINE on STN  
 AN 90264431 MEDLINE  
 TI Purification and biochemical characterization of SELB, a translation factor involved in selenoprotein synthesis.  
 AU Forchhammer K; Rucknagel K P; Bock A  
 SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1990 Jun 5) 265 (16) 9346-50.  
 Journal code: 2985121R. ISSN: 0021-9258.
- AB The product of the selB gene from Escherichia coli is required for co-translational insertion of selenocysteine into protein. To make the SELB protein accessible to biochemical analysis, the protein was purified from cells that overexpressed the selB gene from a phage T7 promoter plasmid. It was calculated that the overproduced SELB protein was purified 20-fold. The N-terminal amino acid sequence of the purified protein was determined, and it confirmed that the initiation codon of selB mRNA translation overlaps the stop codon of the preceding selA gene by 4 bases. Structural similarity between SELB and elongation factors was demonstrated by limited proteolysis of SELB by trypsin. The cleavage sites within SELB were identified by N-terminal sequencing of the two proteolytic products. The position in the SELB protein of the major cleavage site was homologous to a tryptic cleavage site which is characteristic for elongation factors. Immunological analysis showed that the levels of SELB are equivalent in aerobically and anaerobically grown cells; the amount of the protein was estimated to be approximately 1100 copies/E. coli cell. Upon fractionation of cell extracts, SELB was found to be partially associated with the ribosomes. The results therefore indicate that SELB is the first known elongation factor-like protein that has specificity for a particular charged tRNA.
- L25 ANSWER 21 OF 23 MEDLINE on STN  
 AN 90228368 MEDLINE  
 TI Selenomethionyl proteins produced for analysis by multiwavelength anomalous diffraction (MAD): a vehicle for direct determination of three-dimensional structure.  
 AU Hendrickson W A; Horton J R; LeMaster D M  
 SO EMBO JOURNAL, (1990 May) 9 (5) 1665-72.  
 Journal code: 8208664. ISSN: 0261-4189.
- AB An expression system has been established for the incorporation of selenomethionine into recombinant proteins produced from plasmids in Escherichia coli. Replacement of methionine by selenomethionine is demonstrated at the level of 100% for both T4 and E. coli thioredoxins. The natural recombinant proteins and the selenomethionyl variants of both thioredoxins crystallize isomorphously. Anomalous scattering factors were deduced from synchrotron X-ray absorption measurements of crystals of the selenomethionyl proteins. Taken with reference to experience in the structural analysis of selenobiotinyl streptavidin by the method of multiwavelength anomalous diffraction (MAD), these data indicate that recombinant selenomethionyl proteins analyzed by MAD phasing

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offer a rather general means for the elucidation of atomic structures.

- L25 ANSWER 22 OF 23 MEDLINE on STN  
AN 85060498 MEDLINE  
TI Occurrence and characterization of selenocysteine in proteins.  
AU Stadtman T C  
SO METHODS IN ENZYMOLOGY, (1984) 107 576-81.  
Journal code: 0212271. ISSN: 0076-6879.
- L25 ANSWER 23 OF 23 MEDLINE on STN  
AN 84058808 MEDLINE  
TI New biologic functions--selenium-dependent nucleic acids and proteins.  
AU Stadtman T C  
SO FUNDAMENTAL AND APPLIED TOXICOLOGY, (1983 Sep-Oct) 3 (5) 420-3.  
Journal code: 8200838. ISSN: 0272-0590.
- AB Selenium occurs normally in living things as a highly specific component of certain enzymes and amino acid transfer nucleic acids (tRNAs). In bacteria, biosynthesis of essential selenoenzymes has been shown to be unaffected by wide variations in sulfur levels. The naturally occurring selenoenzymes so far identified from bacterial sources include glycine reductase, certain formate dehydrogenases, a hydrogenase, nicotinic acid hydroxylase, xanthine dehydrogenase and thiolase. The selenoenzyme, glutathione peroxidase, and three other selenoproteins of unknown function have been isolated from animals. In certain enzymes, e.g. glycine reductase, formate dehydrogenase, hydrogenase and glutathione peroxidase, the chemical form of selenium has been identified as selenocysteine. One enzyme, a bacterial thiolase, contains selenomethionine rather than selenocysteine. A labile, unidentified form of selenium is present in nicotinic acid hydroxylase, and by inference, xanthine dehydrogenase. The seleno-tRNAs serve as examples of a different type of biological macromolecule that is specifically modified with selenium. The major seleno-tRNAs in *Clostridium sticklandii* and *Escherichia coli* have been identified as glutamate and lysine isoaccepting species. The selenium-modified nucleoside is 5-methyl-aminomethyl-2-selenouridine (mnm5Se2U), which is the chemical analog of 5-methylaminomethyl-2-thiouridine, a previously identified minor base of *E. coli* tRNA<sup>2</sup>Glu. The seleno-tRNA<sup>Glu</sup> of *C. sticklandii* contains one gram atom of Se per mole of biologically active tRNA. Loss of Se from the modified nucleoside, mnm5Se2U, in this tRNA results in concomitant loss of glutamate charging activity suggesting that selenium is essential for interaction of the synthetase and its cognate tRNA.

(FILE 'HCAPLUS' ENTERED AT 15:51:16 ON 24 OCT 2003)

- L1 5 SEA FILE=REGISTRY ABB=ON PLU=ON MERCURY/CN OR "MERCURY (HG2)"/CN OR ("MERCURY (HG2+)")/CN OR "MERCURY (HG22+)"/CN OR "MERCURY (HG4)"/CN)
- L2 1 SEA FILE=REGISTRY ABB=ON PLU=ON PLATINUM/CN
- L3 6 SEA FILE=REGISTRY ABB=ON PLU=ON (IODINE/CN OR "IODINE (127I2)"/CN OR "IODINE (129I2)"/CN OR "IODINE (I1+)"/CN OR "IODINE (I129I)"/CN OR "IODINE (I2-)"/CN OR "IODINE (I21+)"/CN)
- L4 7 SEA FILE=REGISTRY ABB=ON PLU=ON IRON/CN OR ("IRON (FE1+)"/CN OR "IRON (FE13+)"/CN OR "IRON (FE2)"/CN OR "IRON (FE2+)"/CN OR "IRON (FE24+)"/CN OR "IRON (FE3+)"/CN

09/989974

)  
L5 11 SEA FILE=REGISTRY ABB=ON PLU=ON (SELENIUM/CN OR  
"SELENIUM (76SE2)"/CN OR "SELENIUM (80SE2)"/CN OR  
"SELENIUM (SE2)"/CN OR "SELENIUM (SE21+)"/CN OR "SELENIUM  
(SE3)"/CN OR "SELENIUM (SE4)"/CN OR "SELENIUM (SE5)"/CN  
OR "SELENIUM (SE6)"/CN OR "SELENIUM (SE7)"/CN OR  
"SELENIUM (SE8)"/CN)  
L6 30 SEA FILE=REGISTRY ABB=ON PLU=ON L1 OR L2 OR L3 OR L4  
OR L5  
L33 203544 SEA FILE=HCAPLUS ABB=ON PLU=ON (POLYPROTEIN OR PROTEIN  
OR PEPTIDE OR POLYPEPTIDE) (5A) (PRODUCE# OR PRODUCING OR  
PRODUCTION OR MANUF? OR SYNTHES?)  
L34 4206 SEA FILE=HCAPLUS ABB=ON PLU=ON L33 AND (L6 OR MERCURY  
OR HG OR PLATINUM OR PT OR IODINE OR IRON OR FE OR  
SELENIUM OR SE(S)SELEN?)  
L35 32 SEA FILE=HCAPLUS ABB=ON PLU=ON L34 AND (COLI OR  
BACTERI## OR YEAST) (10A) (EXTRACT? OR EXT##)  
L40 12 SEA FILE=HCAPLUS ABB=ON PLU=ON L35 AND (NUCLEIC OR DNA  
OR DEOXYRIBONUCLEIC OR DEOXY RIBONUCLEIC OR AMINO)  
L41 11 L40 NOT L15

L41 ANSWER 1 OF 11 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2002:634340 HCAPLUS

DOCUMENT NUMBER: 137:153943

TITLE: Method for the fermentative production of  
O-acetyl-L-serine

INVENTOR(S): Maier, Thomas; Dassler, Tobias; Boeck, August

PATENT ASSIGNEE(S): Consortium Fuer Elektrochemische Industrie Gmbh,  
Germany

SOURCE: Eur. Pat. Appl., 10 pp.

CODEN: EPXXDW

DOCUMENT TYPE: Patent

LANGUAGE: German

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 1233067	A1	20020821	EP 2002-2036	20020207
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
DE 10107002	A1	20020829	DE 2001-10107002	20010215
JP 2002262896	A2	20020917	JP 2002-35690	20020213
US 2002146783	A1	20021010	US 2002-77022	20020214
US 6620598	B2	20030916		
CN 1370836	A	20020925	CN 2002-104769	20020219
PRIORITY APPLN. INFO.:			DE 2001-10107002 A	20010215

OTHER SOURCE(S): CASREACT 137:153943

AB A fermentation process is provided for the production of O-acetyl-L-serine from a microbial wild type strain or a derivative that has elevated endogenous O-acetyl-L-serine concns. Also provided is the cultivation of said microbial strain such that the intracellular O-acetyl-L-serine is transported out of the cells. Said process is preformed in a fermentation medium at pH 5.1-6.5. Thus, Escherichia strain W3110/pA-CYC184-cysEX-GAPDH-ORF306, which possesses a feedback resistant serine acetyltransferase gene (ydeD), was cultivated in a fed-batch fermentation using a medium containing dextrose, tryptone,

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**yeast extract**, salts, vitamins, and trace elements.  
After 28 h, 9.0 g/L O-acetyl-L-serine was produced along with 4.2 g/L N-acetyl-L-serine and 1,9 g/L 2-methyl-2,4-thiazolidinedicarboxylic acid.

IT **7439-89-6D, Iron**, salts of

RL: BCP (Biochemical process); BIOL (Biological study); PROC (Process)

(method for fermentative production of O-acetyl-L-serine)

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L41 ANSWER 2 OF 11 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2002:416515 HCAPLUS

DOCUMENT NUMBER: 136:381368

TITLE: Recombinant production and purification of human selenoprotein P gene

INVENTOR(S): Yang, Jianguo

PATENT ASSIGNEE(S): Beijing City Nutritive Source Inst., Peop. Rep. China

SOURCE: Faming Zhuanli Shenqing Gongkai Shuomingshu, 9 pp.  
CODEN: CNXXEV

DOCUMENT TYPE: Patent

LANGUAGE: Chinese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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CN 1314469	A	20010926	CN 2000-103136	20000320

PRIORITY APPLN. INFO.: CN 2000-103136 20000320

AB The process comprises isolation of selenoprotein P gene from human hepatocytes by RP-PCR with special primer. The invention also relates to construction of expression plasmid vector for selenoprotein P and expression of selenoprotein P in E. coli. The invention also provides contents, such as **yeast extract**, peptone, Na<sub>2</sub>SeO<sub>3</sub> and NaCl, of culture media for fermentation of selenoprotein P. The invention further provides methods for purification of selenoprotein P by ion-exchange chromatog. and mol. sieves.

L41 ANSWER 3 OF 11 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2001:921138 HCAPLUS

DOCUMENT NUMBER: 136:231319

TITLE: **Production** of single-cell **protein** from ram horn hydrolysate

AUTHOR(S): Kurbanoglu, Esabi Basaran

CORPORATE SOURCE: Science and Letters Faculty, Department of Biology, Ataturk University, Erzurum, 25240, Turk.

SOURCE: Turkish Journal of Biology (2001), 25(4), 371-377

CODEN: TJBIEZ; ISSN: 1300-0152

PUBLISHER: Scientific and Technical Research Council of Turkey

DOCUMENT TYPE: Journal

LANGUAGE: English

Searcher : Shears 308-4994

- AB *Candida utilis* NRRL Y-900 was grown on horn hydrolyzate for single-cell **protein production**. First, ram horns obtained from slaughterhouse in Erzurum were hydrolyzed by phys. and chemical methods and crude horn hydrolyzate (CHH) was obtained. The contents of protein, nitrogen, ash, some minerals, total sugars and **amino** acids of CHH were determined and it was seen that it has sufficient organic and inorg. materials to allow its use as a substrate source in the **production** of single-cell **protein**. The CHH was enriched by addition of **yeast extract**, glucose and KH<sub>2</sub>PO<sub>4</sub>. The effects of different CHH concns. (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10%) on the growth of *C. utilis* were investigated and 4% of CHH (Horn Broth=HB) was found to be optimal. The biomass yield of *C. utilis* and its protein content were found to be 6.8 g l<sup>-1</sup> and 49.8% resp. On the other hand, biomass contained 5.4% lipids, 5.94% RNA, 1.53% **DNA** and 9.7% ash. The biomass contained all of the essential **amino** acids and when compared with FAO reference protein it showed a good profile. The results demonstrated that ram horns can be used as a substrate source in the **production** of single-cell **protein**.
- IT 7439-89-6, **Iron**, biological studies  
 RL: BSU (Biological study, unclassified); BIOL (Biological study) (production of single-cell **protein** from ram horn hydrolyzate)

REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L41 ANSWER 4 OF 11 HCAPLUS COPYRIGHT 2003 ACS on STN  
 ACCESSION NUMBER: 2001:470933 HCAPLUS  
 DOCUMENT NUMBER: 135:207984  
 TITLE: Involvement of a transformylase enzyme in siderophore synthesis in *Pseudomonas aeruginosa*  
 AUTHOR(S): McMorran, Brendan J.; Kumara, H. M. C. Shantha; Sullivan, Kate; Lamont, Iain L.  
 CORPORATE SOURCE: Department of Biochemistry and Centre for Gene Research, University of Otago, Dunedin, N. Z.  
 SOURCE: Microbiology (Reading, United Kingdom) (2001), 147(6), 1517-1524  
 CODEN: MROBEO; ISSN: 1350-0872  
 PUBLISHER: Society for General Microbiology  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English

- AB Fluorescent pseudomonads produce yellow-green siderophores when grown under conditions of **Fe** starvation. Here, the characterization of the pvdF gene, which is required for synthesis of the siderophore pyoverdine by *P. aeruginosa* strain PAO1, is described. A *P. aeruginosa* pvdF mutant was constructed and found to be defective for production of pyoverdine, demonstrating the involvement of PvdF in pyoverdine synthesis. Transcription anal. showed that expression of pvdF was regulated by the amount of **Fe** in the growth medium, consistent with its role in siderophore production. **DNA** sequencing showed that pvdF gives rise to a protein of 31 kDa that has similarity with glycinamide ribonucleotide transformylase (GART) enzymes involved in purine synthesis from a wide range of eukaryotic and prokaryotic species. Chemical analyses of **exts.** from wild-type and pvdF mutant **bacteria** indicated that the PvdF enzyme catalyzes the formylation of N5-hydroxyornithine to give rise to N5-formyl-N5-hydroxyornithine, a

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component of pyoverdine. These studies enhance understanding of the enzymol. of pyoverdine synthesis, and to the best of the authors' knowledge provide the 1st example of involvement of a GART-type enzyme in synthesis of a secondary metabolite.

REFERENCE COUNT: 59 THERE ARE 59 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L41 ANSWER 5 OF 11 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2000:711388 HCAPLUS

DOCUMENT NUMBER: 134:99614

TITLE: Reaction engineering studies for the production of extracellular human calcitonin-precursors with Staphylococcus carnosus

AUTHOR(S): Dilsen, Susanne

CORPORATE SOURCE: Forschungszentrum Julich, Institut fur Biotechnologie, Germany

SOURCE: Berichte des Forschungszentrums Juelich (2000), Juel-3765, i-vi, 1-143

CODEN: FJBEE5; ISSN: 0366-0885

DOCUMENT TYPE: Report

LANGUAGE: German

AB This study presents the development of a fedbatch process for the **production** of secreted heterologous **proteins** using the Gram-pos. bacterium Staphylococcus carnosus up to a semi-tech. scale. The model protein was human calcitonin which was expressed and secreted by S. carnosus as a fusion protein consisting of a propeptide from a lipase of Staphylococcus hyicus and a calcitonin dimer. Based on investigations on the influences of different C-, N-, and energy-sources on growth and product formation a process applying **yeast extract** limiting conditions (closed-loop substrate control) was established using the pH as a supporting value. Means of this process 2 g L-1 of the fusion **protein** were **produced** in a semi-tech. scale (150 l) comprising a space-time-yield of 142 mg L-1 h-1. The **yeast extract** specific protein-yield amounted to 40 mg g-1 for a tech. **yeast extract**

IT 7439-89-6, Iron, biological studies

RL: BAC (Biological activity or effector, except adverse); BSU

(Biological study, unclassified); BIOL (Biological study)

(medium optimization for calcitonin production by Staphylococcus carnosus)

REFERENCE COUNT: 80 THERE ARE 80 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L41 ANSWER 6 OF 11 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 1999:770108 HCAPLUS

DOCUMENT NUMBER: 133:27206

TITLE: Expression of cloned human lactoferrin cDNA in transgenic tobacco confers resistance to bacterial and viral diseases

AUTHOR(S): Zhang, Da-Bing; Zhang, Jing-Liu; Wang, Zong-Yang; Hong, Meng-Min

CORPORATE SOURCE: Shanghai Institute of Plant Physiology, Chinese Academy of Sciences, Shanghai, 200032, Peop. Rep. China

SOURCE: Zhiwu Shengli Xuebao (1999), 25(3), 234-243

PUBLISHER: Kexue Chubanshe  
 DOCUMENT TYPE: Journal  
 LANGUAGE: Chinese

AB Lactoferrin is an **iron**-binding glycoprotein present in human milk. One of its functions is the inhibition of bacterial growth. It has broad-spectrum antimicrobial properties and is widely considered to be an important component of the host defense against microbial infections. To investigate if lactoferrin gene could express in plant cell and confer resistance on transgenic plants to some pathogens, the cDNA encoding human lactoferrin was cloned and introduced into tobacco cells. The preliminary results are as follows. The total cellular RNAs were isolated from human neutrophil cells, and the first-strand cDNA was prepared from 1 µg of total cellular RNAs by oligo(dT)-primed **DNA** synthesis with AMV reverse transcriptase. Two doubled-stranded lactoferrin cDNA fragments of 1 372 bp (from 1 to 1 372 nucleotide) and 928 bp (from 1 353 to 2 288 nucleotide) in length, resp., were synthesized from the first cDNA products by amplification using Nest-PCR strategy(Fig. 2). Then, full length of the 2 288 bp cDNA of lactoferrin ligated from these two **DNA** fragments was obtained. Results of nucleotide anal. showed that the sequence in the coding region was identical to that given in Database, however, three nucleotides differences were found to be present in the 3'-untranslated end. The coding region of lactoferrin cDNA was inserted in the downstream of CaMV 35S promoter within the Ti plasmid pBI121 to construct lactoferrin expressing plasmid p35ShLFC (Fig. 1). Transformation of *Nicotiana tabacum* var. Gexin Number 1 was performed using the leaf disk procedure. After inoculation with *A. tumefaciens* strain LBA4404 harboring p35ShLFC plasmid, the leaf tissue was cultivated on MS medium supplemented with Cef, Cb and 2,4-D. Shoots growing on this medium were transferred to MS medium containing the same antibiotics but no hormones to allow rooting. Km-resistant plants were transferred to the greenhouse, where F1 plants were produced by selfing. The stable integration of the lactoferrin cDNA into the genome of transgenic tobacco was demonstrated by **DNA** gel blot hybridization and PCR anal. (Fig. 3). The lactoferrin cDNA was used as a probe which did not hybridize to **DNA** from untransformed control, but identified a BamH I -Sac I restriction fragment of the expected length in genomic **DNA** isolated from 5 transformants. To detect the transcript of lactoferrin cDNA in the transgenic tissues, total RNAs were extracted from leaves of transgenic tobacco plants, and a probe used was the same as that used in Southern blot anal. The results showed that hybridization band was detected in transgenic progeny plants, but not in leaves of untransformed control tobacco plant(Fig. 4). To detect the **production** of lactoferrin **protein** in transgenic plants, a poly-cloned antiserum was raised against human lactoferrin purchased from Sigma Co. Results of Western blot anal. showed that a protein band with mol. mass 50 kD in the extract from transgenic tobacco was detected on SDS-PAGE gel (Fig. 5). This mol. mass is about 30 kD lower than that for human milk lactoferrin (80 kD). Differences in mol. mass of lactoferrin between the two sources are probably due to the difference in mol. size of the glycosylation moiety and proteolytic enzyme digestion within tobacco cells. However, the protein **extracted** from transgenic tobacco plants exhibited antibacterial activity against phytopathogenic **bacteria** *Pseudomonas syringae* pv. *tabaci*



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in vitro and in vivo (Fig. 6). In addition, transgenic plants could alleviate mosaic symptoms in tobacco leaves inoculated with tobacco mosaic virus (Fig. 7).

L41 ANSWER 7 OF 11 HCAPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 1999:698939 HCAPLUS  
DOCUMENT NUMBER: 131:335937  
TITLE: Manufacture of hydrogen with a Rhodobacter  
sphaeroides mutant unable to accumulate  
polyhydroxyalkanoate  
INVENTOR(S): Franchi, Elisabetta; Toggi, Claudio; Scolla,  
Giuseppe; Rodriguez, Francesco; Pedroni, Paola  
PATENT ASSIGNEE(S): Zaidan Hojin Chikyu Kankyo Sangyo Gijutsu Kenkyu  
Kiko, Italy; Eniricerche S.p.A.  
SOURCE: Jpn. Kokai Tokkyo Koho, 18 pp.  
CODEN: JKXXAF  
DOCUMENT TYPE: Patent  
LANGUAGE: Japanese  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 11299492	A2	19991102	JP 1999-31475	19990209
JP 3169927	B2	20010528		
IT 1300062	B1	20000419	IT 1998-MI810	19980417
IT 98MI0810	A1	19991018		

PRIORITY APPLN. INFO.: IT 1998-MI810 A 19980417

AB Described are a mutant strain of Rhodobacter sphaeroides RV (CBS100467) which is unable to accumulate polyhydroxyalkanoate (PHA), a method of producing H by anaerobic cultivation of the mutant under illumination, and a culture medium composition for the production. The mutant is cultivated under illumination ranged 6,000-20,000 lx, at 25-40°, pH .5-8.0, in a defined medium containing C and N sources, cations, and anions. The mutant strain SMV071 was prepared by using an interposon containing a kanamycin resistance expression cassette to inactivate gene phaC that encodes PHA synthase. The mutant was able to produce 26% more H than the wild type.

IT 7439-89-6, Iron, biological studies

RL: BUU (Biological use, unclassified); BIOL (Biological study);  
USES (Uses)

(culture medium composition; manufacture of hydrogen with a Rhodobacter sphaeroides mutant unable to accumulate polyhydroxyalkanoate)

L41 ANSWER 8 OF 11 HCAPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 1996:730849 HCAPLUS  
DOCUMENT NUMBER: 126:15499  
TITLE: Specific binding of the Listeria monocytogenes  
transcriptional regulator PrfA to target  
sequences requires additional factors(s) and is  
influenced by iron  
AUTHOR(S): Bockmann, Regine; Dickneite, Carmen; Middendorf,  
Barbara; Goebel, Werner; Sokolovic, Seljka  
CORPORATE SOURCE: Theodor-Boveri-Inst. fuer Biowissenschaften,  
Univ. Wuerzburg, Wuerzburg, 97074, Germany  
SOURCE: Molecular Microbiology (1996), 22(4), 643-653  
CODEN: MOMIEE; ISSN: 0950-382X

Searcher : Shears 308-4994

09/989974

PUBLISHER: Blackwell  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB The PrfA protein, which is a member of the Crp/Fnr family of prokaryotic transcription activators, regulates the virulence genes of *Listeria monocytogenes*. In this work, specific binding of PrfA to its target **DNA** was determined by electrophoretic mobility-shift assays (EMSAs) using cell-free exts. from the two *L. monocytogenes* strains EGD and NCTC 7973. PrfA-specific binding differs between the two strains, even when the concentration of PrfA was adjusted to similar levels. Both strains exhibited increased PrfA-specific binding after a shift into minimal essential medium (MEM) without showing a significant change in the amount of PrfA protein, relative to exts. from **bacteria** grown in brain-heart infusion (BHI). The purified PrfA **protein** from strain EGD **produced** in *Escherichia coli* did not exhibit specific binding to the target **DNA** but did so upon addition of PrfA-free exts. from various *Listeria* species and *Bacillus subtilis*. The observed activation of PrfA seems to be caused by a PrfA-activating factor (Paf), which is probably a protein since elevated temperature, but not RNase treatment, destroyed the activation potential of such PrfA-free exts. Moreover, fractionation of these exts. by sucrose gradient centrifugation yielded the Paf activity in a fraction sedimenting at 3.2S. Specific binding of PrfA-containing exts. from strain EGD to the *hly* and *actA* promoter sequences was strongly inhibited by **iron**, whereas that of exts. from strain NCTC 7973 was only slightly reduced. The **iron** effect seems to be mediated by Paf rather than by PrfA itself.

IT **7439-89-6, Iron**, biological studies  
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)  
(binding of *Listeria monocytogenes* transcriptional regulator PrfA to target sequences requires addnl. factors(s) and is influenced by **iron**)

L41 ANSWER 9 OF 11 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 1995:906988 HCAPLUS

DOCUMENT NUMBER: 123:309436

TITLE: Structural and immunological studies on the soluble formate dehydrogenase from *Alcaligenes eutrophus*

AUTHOR(S): Friedebold, Joerg; Mayer, Frank; Bill, Eckhard; Trautwein, Alfred X.; Bowien, Botho

CORPORATE SOURCE: Inst. Mikrobiologie, Georg-August-Univ., Goettingen, D-37077, Germany

SOURCE: Biological Chemistry Hoppe-Seyler (1995), 376(9), 561-8

CODEN: BCHSEI; ISSN: 0177-3593

PUBLISHER: de Gruyter

DOCUMENT TYPE: Journal

LANGUAGE: English

AB During growth with formate as the sole energy source the autotrophic bacterium *Alcaligenes eutrophus* synthesizes a cytoplasmic formate dehydrogenase. The enzyme is a molybdo-**iron**-sulfur-flavo **protein** and the major NADH-**producing** system under these growth conditions, although it was estimated to constitute only 0.65% of the soluble cell protein. An electron microscopic anal. of the purified enzyme revealed that the particle is made up of four

nonidentical submasses, corroborating previous structural data. The NH<sub>2</sub>-terminal **amino** acid sequences of the enzyme subunits exhibited significant similarities to those of only one other heteromeric formate dehydrogenase, the enzyme from the methane-utilizing bacterium *Methylosinus trichosporium*. Metal analyses yielded 21.5 g-atom **iron**, 2.18 g-atom nickel, 0.76 g-atom molybdenum, and 0.59 g-atom zinc per mol of enzyme. Initial ESR spectroscopic studies showed at least three distinct signals which appeared upon reduction of the enzyme with NADH or formate. The corresponding spin systems could be attributed to **iron-sulfur** centers of the enzyme. Comparative immunostaining and activity-staining expts. using cell **exts** . from various **bacteria** established immunol. similarities between the soluble formate dehydrogenase of *A. eutrophus* and the soluble enzymes from all tested facultative autotrophs as well as from *M. trichosporium*.

IT **7439-89-6, Iron**, biological studies  
 RL: BOC (Biological occurrence); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence)  
 (structural and immunol. studies on the soluble formate dehydrogenase from *Alcaligenes eutrophus*)

L41 ANSWER 10 OF 11 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 1978:188152 HCAPLUS  
 DOCUMENT NUMBER: 88:188152  
 TITLE: Functional yeast proteins  
 INVENTOR(S): Chao, Kwei C.  
 PATENT ASSIGNEE(S): Standard Oil Co. (Indiana), USA  
 SOURCE: Ger. Offen., 20 pp.  
 CODEN: GWXXBX  
 DOCUMENT TYPE: Patent  
 LANGUAGE: German  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DE 2738356	A1	19780316	DE 1977-2738356	19770825
US 4079048	A	19780314	US 1976-722050	19760910
CA 1087445	A1	19801014	CA 1977-283101	19770719
NL 7709071	A	19780314	NL 1977-9071	19770817
JP 53034992	A2	19780331	JP 1977-100388	19770822
JP 56050560	B4	19811130		
BE 858409	A1	19780306	BE 1977-180679	19770905
FR 2363996	A1	19780407	FR 1977-27114	19770907
FR 2363996	B1	19810508		
ES 462255	A1	19781201	ES 1977-462255	19770909
GB 1578235	A	19801105	GB 1977-37749	19770909

PRIORITY APPLN. INFO.: US 1976-722050 19760910

AB A protein preparation with useful foam-forming and emulsifying properties is prepared from yeast. The **yeast** cells are 1st **extracted** with a hot aqueous solution, then separated from the extract and suspended in a hot dilute alkaline solution, pH 8.5-10, at 85-95° to remove **nucleic** acid material. The cells are then suspended in 0.1-0.3 N alkali at 85-100° to extract protein. The extract is neutralized to precipitate undigested material and the supernatant is acidified to precipitate protein at the isoelec. **pt** . The mother liquor is then dried to **produce** the

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**protein.**

L41 ANSWER 11 OF 11 HCAPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 1974:118103 HCAPLUS  
DOCUMENT NUMBER: 80:118103  
TITLE: Synthesis of diphtheria tox-gene products in  
Escherichia coli extracts  
AUTHOR(S): Murphy, J. R.; Pappenheimer, A. M., Jr.; Tayart  
de Borms, S.  
CORPORATE SOURCE: Biol. Lab., Harvard Univ., Cambridge, MA, USA  
SOURCE: Proceedings of the National Academy of Sciences  
of the United States of America (1974), 71(1),  
11-15  
CODEN: PNASA6; ISSN: 0027-8424  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB In a **protein-synthesizing** system **extracted**  
from E. coli, purified **DNA** from corynephages  
 $\beta$ ctox+ and  $\beta$ 45c was used to direct in vitro synthesis of  
diphtheria toxin and related nontoxic protein, CRM45, as well as  
other  $\beta$ -phage proteins. When  $\beta$ ctox+- **DNA** or  
 $\beta$ -45c- **DNA** was added to a similar system extracted from  
the nonlysogenic Corynebacterium diphtheriae strain, C7s(-)tox-,  
neither toxin nor the CRM45 **protein** was **produced**  
, although other  $\beta$ -phage **proteins** were  
**synthesized** in amts. equivalent to those produced in the E. coli  
system from the same amount of  $\beta$ -phage **DNA**.  
Preliminary expts. suggest that both toxinogenic and nontoxinogenic  
strains of the diphtheria bacillus contain a factor that  
specifically blocks expression of the tox gene. **Synthesis**  
of toxin and the CRM45 **protein** in the E. coli system was  
not inhibited by inorg. **Fe**, but was inhibited by exts.  
from the C7s(-)tox- strain of C. diphtheriae.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH,  
JICST-EPLUS, JAPIO' ENTERED AT 15:52:21 ON 24 OCT 2003)

L42 25 S L40  
L43 25 S L42 NOT L16  
L44 16 DUP REM L43 (9 DUPLICATES REMOVED)

L44 ANSWER 1 OF 16 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS  
RESERVED. on STN DUPLICATE 1  
ACCESSION NUMBER: 2002357921 EMBASE  
TITLE: Proteomic analysis of a thermostable superoxide  
dismutase from Bacillus stearothermophilus TLS33.  
AUTHOR: Sookkheo B.; Sinchaikul S.; Thannan H.; Thongprasong  
O.; Phutrakul S.; Chen S.-T.  
CORPORATE SOURCE: Dr. S.-T. Chen, Institute of Biological Chemistry,  
Academia Sinica, 128 Yen Chiu Yuan Rd., Nankang,  
Taipei 11529, Taiwan, Province of China.  
bcchen@gate.sinica.edu.tw  
SOURCE: Proteomics, (1 Sep 2002) 2/9 (1311-1315).  
Refs: 29  
ISSN: 1615-9853 CODEN: PROTC7  
COUNTRY: Germany  
DOCUMENT TYPE: Journal; Conference Article  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: English

09/989974

SUMMARY LANGUAGE: English

AB Thermophilic bacterium *Bacillus stearothermophilus* TLS33 isolated from a hot spring in Chiang Mai, Thailand produces an extracellular superoxide dismutase (SOD). SOD is a free radical metabolizing enzyme that protects the cell membrane from damage by the highly reactive superoxide free radicals. To identify the secreted SOD, we used the systematically proteomic approaches of two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) analysis and database searching. The **bacterium** was grown in a medium containing 0.1% w/v **yeast extract** and 0.1% w/v tryptone in 100% v/v base mixture at 65°C for 72 h, by assessing their growth by **protein** and SOD activity. The bacterium **produced** the highest SOD activity at 65°C for 48 h and the extracellular SOD was run on 2-D PAGE using broad range pH 3-10 immobilized pH gradients (IPGs) and narrow range pH 4-7 IPGs. The isoelectric point and molecular mass of the extracellular SOD were approximately 5.8 and 28 kDa, respectively. In addition, the NH2-terminal **amino** acid sequence was found to be P-F-E-L-P-A-L-P-Y-P-Y-D-A-L-E-P-P-I-I-D, which had a homology of approximately 85% to the Mn-SOD family and 65% to the **Fe**-SOD family.

L44 ANSWER 2 OF 16 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN  
ACCESSION NUMBER: 2000-023264 [02] WPIDS  
DOC. NO. CPI: C2000-005641  
TITLE: Isolated surface attachment defective genes, useful  
for developing products which modulate bacterial  
biofilm formation.  
DERWENT CLASS: B04 D16 D22  
INVENTOR(S): KOLTER, R; O'TOOLE, G A; PRATT, L  
PATENT ASSIGNEE(S): (HARD) HARVARD COLLEGE  
COUNTRY COUNT: 21  
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9955368	A1	19991104	(200002)*	EN	151
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE					
W: CA US					
EP 1079861	A1	20010307	(200114)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9955368	A1	WO 1999-US9034	19990427
EP 1079861	A1	EP 1999-920038	19990427
		WO 1999-US9034	19990427

FILING DETAILS:

PATENT NO	KIND	PATENT NO
EP 1079861	A1 Based on	WO 9955368

PRIORITY APPLN. INFO: US 1998-102870 19981002; US 1998-83259  
19980427

Searcher : Shears 308-4994

AN 2000-023264 [02] WPIDS  
 AB WO 9955368 A UPAB: 20000112

NOVELTY - **Nucleic** acids comprising at least 75 consecutive nucleotides that form a region that hybridizes to a probe comprising at least 75 consecutive nucleotides complementary to a portion of an n-sad (surface attachment defective) gene, are new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a probe comprising at least 18 nucleotides complementary to a *Pseudomonas fluorescens* n-sad gene comprising a sequence of sequences 1090, 277, 819, 832, 1054, 880, 779, 848, 533, 591, 1249, 373, 683, 672, 1676, 721, 452, 442, 538, 2118, 642, 583, 360 and 494 nucleotides ((I)-(XXIV) respectively; given in the specification);

(2) a pure n-sad polypeptide;

(3) a pure antibody that specifically binds an n-Sad polypeptide;

(4) screening for a compound that modulates biofilm formation by:

(a) contacting a sample containing a sad gene, a sad/reporter gene, or a Sad polypeptide with a test compound; and

(b) measuring the level of sad activity in the sample;

(5) screening for a compound that modulates biofilm formation by:

(a) contacting a sample containing a clpP gene, a clpP/reporter gene, or a ClpP polypeptide with a test compound; and

(b) measuring the level of ClpP activity in the sample;

(6) preventing a bacterial cell from participating in the formation of a biofilm, by:

(a) inhibiting the **synthesis** or function of a Sad polypeptide;

(b) inhibiting **protein synthesis** in the cell;

(c) contacting the cell with a protease;

(d) limiting the concentration of Fe<sup>2+</sup> and/or Fe<sup>3+</sup> in the cells environment to at most 0.3 micro M;

(e) placing the cell in an environment with an osmolarity at least equal to that of a solution containing 0.2 M NaCl or 15 % sucrose; or

(f) adding mannose or alpha -methyl-D-mannoside to the cells environment to a concentration of at least 15 mM;

(7) inhibiting the participation of a bacterium in formation of a biofilm on an optionally abiotic surface, by inhibiting the synthesis or function of flagella, or pila on the bacterium;

(8) screening for a compound that inhibits bacterial pathogenicity by:

(a) exposing a bacterial culture to a test compound; and

(b) testing the bacterial culture for biofilm formation on an abiotic surface;

(9) stimulating the formation of a biofilm by a population of bacteria by:

(a) adding **iron** to the bacterial growth environment to a final concentration of at least 3 micro M;

(b) adding glutamate or citrate to the growth environment to a final concentration of at least 0.4%; and

(c) stimulating the expression of a sad gene or activity of a Sad polypeptide.

ACTIVITY - Antibacterial.

MECHANISM OF ACTION - Cell-cell binding modulation.

USE - The sad genes, gene products, and transcriptional control regions may all be used for the control of biofilm formation in fields such as manufacturing, agriculture, and healthcare. The products may also be used to identify compounds which modulate biofilm formation. Compounds that stimulate biofilm formation can be used to improve colonization of plant roots by beneficial bacteria. Conversely, compounds that inhibit biofilm formation can be used to restrict the growth of bacteria on contact lenses, medical implants (e.g. artificial hips), the walls of catheters, fluid contacting surfaces of industrial devices, water and sewerage pipes, and within the lungs of infected patients. The inhibitor compounds can also be applied to soil, seeds or plant roots to inhibit bacterial growth.

Dwg.0/23

L44 ANSWER 3 OF 16 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2000:124408 BIOSIS

DOCUMENT NUMBER: PREV200000124408

TITLE: Expression of cloned human lactoferrin cDNA in transgenic tobacco confers resistance to bacterial and viral diseases.

AUTHOR(S): Zhang Da-Bing [Reprint author]; Zhang Jing-Liu [Reprint author]; Wang Zong-Yang [Reprint author]; Hong Meng-Min [Reprint author]

CORPORATE SOURCE: Shanghai Institute of Plant Physiology, Chinese Academy of Sciences, Shanghai, 200032, China

SOURCE: Acta Phytobiologica Sinica, (1999) Vol. 25, No. 3, pp. 234-243. print.

CODEN: CWSPDA. ISSN: 0257-4829.

DOCUMENT TYPE: Article

LANGUAGE: Chinese

ENTRY DATE: Entered STN: 5 Apr 2000

Last Updated on STN: 3 Jan 2002

AB Lactoferrin is an **iron**-binding glycoprotein present in human milk. One of its functions is the inhibition of bacterial growth. It has broad-spectrum antimicrobial properties and is widely considered to be an important component of the host defense against microbial infections. To investigate if lactoferrin gene could express in plant cell and confer resistance on transgenic plants to some pathogens, the cDNA encoding human lactoferrin was cloned and introduced into tobacco cells. The preliminary results are as follows. The total cellular RNAs were isolated from human neutrophil cells, and the first-strand cDNA was prepared from 1 mug of total cellular RNAs by oligo (dT)-primed **DNA** synthesis with AMV reverse transcriptase. Two doubled-stranded lactoferrin cDNA fragments of 1 372 bp (from 1 to 1 372 nucleotide) and 928 bp (from 1 353 to 2 288 nucleotide) in length, respectively, were synthesized from the first cDNA products by amplification using Nest-PCR strategy (Fig. 2). Then, full length of the 2 288 bp cDNA of lactoferrin ligated from these two **DNA** fragments was obtained. Results of nucleotide analysis showed that the sequence in the coding region was identical to that given in Database, however, three nucleotides differences were found to be present in the 3'-untranslated end. The coding region of lactoferrin cDNA was inserted in the downstream of CaMV 35S promoter within the Ti plasmid pBI121 to construct lactoferrin expressing plasmid p35ShLFC (Fig. 1). Transformation of *Nicotiana tabacum* var. Gexin Number 1 was performed using the leaf disc procedure. After inoculation with A.

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tumefaciens strain LBA4404 harboring p35ShLFC plasmid, the leaf tissue was cultivated on MS medium supplemented with Cef, Cb and 2,4-D. Shoots growing on this medium were transferred to MS medium containing the same antibiotics but no hormones to allow rooting. Km-resistant plants were transferred to the greenhouse, where F1 plants were produced by selfing. The stable integration of the lactoferrin cDNA into the genome of transgenic tobacco was demonstrated by **DNA** gel blot hybridization and PCR analysis (Fig. 3). The lactoferrin cDNA was used as a probe which did not hybridize to **DNA** from untransformed control, but identified a BamH I-Sac I restriction fragment of the expected length in genomic **DNA** isolated from 5 transformants. To detect the transcript of lactoferrin cDNA in the transgenic tissues, total RNAs were extracted from leaves of transgenic tobacco plants, and a probe used was the same as that used in Southern blot analysis. The results showed that hybridization band was detected in transgenic progeny plants, but not in leaves of untransformed control tobacco plant (Fig. 4) To detect the **production** of lactoferrin **protein** in transgenic plants, a polycloned antiserum was raised against human lactoferrin purchased from Sigma Co. Results of Western blot analysis showed that a protein band with molecular mass 50 kD in the extract from transgenic tobacco was detected on SDS-PAGE gel (Fig. 5). This molecular mass is about 30 kD lower than that for human milk lactoferrin (80 kD). Differences in molecular mass of lactoferrin between the two sources are probably due to the difference in molecular size of the glycosylation moiety and proteolytic enzyme digestion within tobacco cells. However, the protein **extracted** from transgenic tobacco plants exhibited antibacterial activity against phytopathogenic **bacteria** Pseudomonas syringae pv. tabaci in vitro and in vivo (Fig. 6). In addition, transgenic plants could alleviate mosaic symptoms in tobacco leaves inoculated with tobacco mosaic virus (Fig. 7).

L44 ANSWER 4 OF 16 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS  
RESERVED. on STN DUPLICATE 2  
ACCESSION NUMBER: 97376677 EMBASE  
DOCUMENT NUMBER: 1997376677  
TITLE: Inhibition of NF- $\kappa$ B **DNA** binding and  
nitric oxide induction in human T cells and lung  
adenocarcinoma cells by selenite treatment.  
AUTHOR: Kim I.Y.; Stadtman T.C.  
CORPORATE SOURCE: T.C. Stadtman, Laboratory of Biochemistry, National  
Heart, Lung/Blood Institute, National Institutes of  
Health, 9000 Rockville Pike, Bethesda, MD 20892,  
United States  
SOURCE: Proceedings of the National Academy of Sciences of  
the United States of America, (1997) 94/24  
(12904-12907).  
Refs: 35  
ISSN: 0027-8424 CODEN: PNAS6  
COUNTRY: United States  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 029 Clinical Biochemistry  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
AB NF- $\kappa$ B is a major transcription factor consisting of 50(p50)-  
and 65(p65)-kDa proteins that controls the expression of various



genes, among which are those encoding cytokines, cell adhesion molecules, and inducible NO synthase (iNOS). After initial activation of NF- $\kappa$ B, which involves release and proteolysis of a bound inhibitor, essential cysteine residues are maintained in the active reduced state through the action of thioredoxin and thioredoxin reductase. In the present study, activation of NF- $\kappa$ B in human T cells and lung adenocarcinoma cells was induced by recombinant human tumor necrosis factor  $\alpha$  or **bacterial** lipopolysaccharide. After lipopolysaccharide activation, nuclear **extracts** were treated with increasing concentrations of **selenite**, and the effects on **DNA**-binding activity of NF- $\kappa$ B were examined. Binding of NF- $\kappa$ B to nuclear responsive elements was decreased progressively by increasing **selenite** levels and, at 7  $\mu$ M **selenite**, **DNA**-binding activity was completely inhibited. **Selenite** inhibition was reversed by addition of a dithiol, DTT. Proportional inhibition of iNOS activity as measured by decreased NO products in the medium (NO<sub>2</sub>- and NO<sub>3</sub>-) resulted from **selenite** addition to cell suspensions. This loss of iNOS activity was due to decreased **synthesis** of NO synthase **protein**. **Selenium** at low essential levels (nM) is required for synthesis of redox active **selenoenzymes** such as glutathione peroxidases and thioredoxin reductase, but in higher toxic levels (>5-10  $\mu$ M) **selenite** can react with essential thiol groups on enzymes to form RS-Se-SR adducts with resultant inhibition of enzyme activity. Inhibition of NF- $\kappa$ B activity by **selenite** is presumed to be the result of adduct formation with the essential thiols of this transcription factor.

L44 ANSWER 5 OF 16 MEDLINE on STN DUPLICATE 3  
 ACCESSION NUMBER: 97109532 MEDLINE  
 DOCUMENT NUMBER: 97109532 PubMed ID: 8951812  
 TITLE: Specific binding of the *Listeria monocytogenes* transcriptional regulator PrfA to target sequences requires additional factor(s) and is influenced by **iron**.  
 AUTHOR: Bockmann R; Dickneite C; Middendorf B; Goebel W; Sokolovic Z  
 CORPORATE SOURCE: Theodor-Boveri-Institut fur Biowissenschaften der Universitat Wurzburg, Germany.  
 SOURCE: MOLECULAR MICROBIOLOGY, (1996 Nov) 22 (4) 643-53. Journal code: 8712028. ISSN: 0950-382X.  
 PUB. COUNTRY: ENGLAND: United Kingdom  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199703  
 ENTRY DATE: Entered STN: 19970414  
 Last Updated on STN: 19970414  
 Entered Medline: 19970331  
 AB The PrfA protein, which is a member of the Crp/Fnr family of prokaryotic transcription activators, regulates the virulence genes of *Listeria monocytogenes*. In this work, specific binding of PrfA to its target **DNA** was determined by electrophoretic mobility-shift assays (EMSAs) using cell-free extracts from the two *L. monocytogenes* strains EGD and NCTC 7973. PrfA-specific binding differs between the two strains, even when the concentration of PrfA

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was adjusted to similar levels. Both strains exhibited increased PrfA-specific binding after a shift into minimal essential medium (MEM) without showing a significant change in the amount of PrfA protein, relative to **extracts** from **bacteria** grown in brain-heart infusion (BHI). The purified PrfA **protein** from strain EGD **produced** in Escherichia coli did not exhibit specific binding to the target **DNA** but did so upon addition of PrfA-free extracts from various Listeria species and Bacillus subtilis. The observed activation of PrfA seems to be caused by a PrfA-activating factor (Paf), which is probably a protein since elevated temperature, but not RNase treatment, destroyed the activation potential of such PrfA-free extracts. Moreover, fractionation of these extracts by sucrose gradient centrifugation yielded the Paf activity in a fraction sedimenting at 3.2 S. Specific binding of PrfA-containing extracts from strain EGD to the hly and actA promoter sequences was strongly inhibited by **iron**, whereas that of extracts from strain NCTC 7973 was only slightly reduced. The **iron** effect seems to be mediated by Paf rather than by PrfA itself.

L44 ANSWER 6 OF 16 MEDLINE on STN DUPLICATE 4  
ACCESSION NUMBER: 96145736 MEDLINE  
DOCUMENT NUMBER: 96145736 PubMed ID: 8561915  
TITLE: Structural and immunological studies on the soluble formate dehydrogenase from Alcaligenes eutrophus.  
AUTHOR: Friedebold J; Mayer F; Bill E; Trautwein A X; Bowien B  
CORPORATE SOURCE: Institut fur Mikrobiologie, Georg-August-Universitat  
Gottigen, Germany.  
SOURCE: BIOLOGICAL CHEMISTRY HOPPE-SEYLER, (1995 Sep) 376 (9)  
561-8.  
Journal code: 8503054. ISSN: 0177-3593.  
PUB. COUNTRY: GERMANY: Germany, Federal Republic of  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199603  
ENTRY DATE: Entered STN: 19960315  
Last Updated on STN: 20000303  
Entered Medline: 19960307

AB During growth with formate as the sole energy source the autotrophic bacterium Alcaligenes eutrophus synthesizes a cytoplasmic formate dehydrogenase. The enzyme is a molybdo-**iron**-sulfur-flavo **protein** and the major NADH-**producing** system under these growth conditions, although it was estimated to constitute only 0.65% of the soluble cell protein. An electron microscopic analysis of the purified enzyme revealed that the particle is made up of four nonidentical submasses, corroborating previous structural data. The NH2-terminal **amino** acid sequences of the enzyme subunits exhibited significant similarities to those of only one other heteromeric formate dehydrogenase, the enzyme from the methane-utilizing bacterium Methylosinus trichosporium. Metal analyses yielded 21.5 g-atom **iron**, 2.18 g-atom nickel, 0.76 g-atom molybdenum, and 0.59 g-atom zinc per mol of enzyme. Initial electron paramagnetic resonance spectroscopic studies showed at least three distinct signals which appeared upon reduction of the enzyme with NADH or formate. The corresponding spin systems could be attributed to **iron**-sulfur centers of the enzyme.

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Comparative immunostaining and activity-staining experiments using cell **extracts** from various **bacteria** established immunological similarities between the soluble formate dehydrogenase of *A. eutrophus* and the soluble enzymes from all tested facultative autotrophs as well as from *M. trichosporium*.

L44 ANSWER 7 OF 16 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN  
ACCESSION NUMBER: 96148689 EMBASE  
DOCUMENT NUMBER: 1996148689  
TITLE: Preparation of **extracts** from **yeast** and the avoidance of proteolysis.  
AUTHOR: Burgers P.M.J.  
CORPORATE SOURCE: Biochemistry/Molec. Biophysics Dept., Washington Univ. School of Medicine, Box 8231, 660 S. Euclid Avenue, St. Louis, MO 63110, United States  
SOURCE: Methods in Molecular and Cellular Biology, (1994) 5/5 (330-335).  
ISSN: 0898-7750 CODEN: MMCBEV  
COUNTRY: United States  
DOCUMENT TYPE: Journal; General Review  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: English  
SUMMARY LANGUAGE: English

AB The yeast *Saccharomyces cerevisiae* is one of the premier organisms for genetic and biochemical studies. However, the isolation of novel factors from yeast or the overproduction of proteins in yeast by overexpression of cloned genes can be seriously complicated by proteolysis during extract preparation and protein purification. Suppression of proteolysis can be achieved by using protease-deficient strains of **yeast** and by including various protease inhibitors during cell breakage and **extract** fractionation. Whole cell **extracts** are prepared by blending **yeast** cells with glass beads. Procedures are described which maximize the yield of nuclear proteins while minimizing proteolytic degradation.

L44 ANSWER 8 OF 16 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 1993:527383 BIOSIS  
DOCUMENT NUMBER: PREV199396140790  
TITLE: Nutritional requirements for synthesis of heat-stable enterotoxin by *Yersinia enterocolitica*.  
AUTHOR(S): Amirmozafari, Nour; Robertson, Donald C. [Reprint author]  
CORPORATE SOURCE: Dep. Bacteriol. Biochem., Univ. Idaho, Moscow, ID 83843, USA  
SOURCE: Applied and Environmental Microbiology, (1993) Vol. 59, No. 10, pp. 3314-3320.  
CODEN: AEMIDF. ISSN: 0099-2240.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 19 Nov 1993  
Last Updated on STN: 3 Jan 1995

AB A defined medium that supported the growth of and synthesis of heat-stable enterotoxin (YST) by clinical isolates of *Yersinia enterocolitica* at levels equivalent to those observed in a complex Trypticase soy broth-0.6% **yeast extract** medium

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was developed. The defined medium contained four **amino** acids (L-methionine, L-glutamic acid, glycine, and L-histidine), inorganic salts, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) buffer, and potassium gluconate as the carbon source. Methionine was required for growth by most strains of *Y. enterocolitica* used in this study; thus, it was not possible to determine whether it was also required for the synthesis of YST. The other 17 **amino** acids commonly found in **proteins** did not stimulate the **synthesis** of YST when added to the defined medium. The yield of YST observed with other carbon sources fermented by *Y. enterocolitica* ranged from 4- to 26-fold lower than that obtained with potassium gluconate. The divalent cations Ca-2+ and Mn-2+ had no effect on the synthesis of YST; however, concentrations of Fe-2+ above 10  $\mu$ M inhibited the synthesis of the enterotoxin. The addition of a mixture of pyrimidines containing thymine, cytosine, and uracil, each at a concentration of 2.0 mM, stimulated the synthesis of YST by 10 to 15%, whereas a mixture of adenine and guanine, each at a similar concentration, inhibited the synthesis of YST. Vitamins had no effect on the amounts of YST produced by *Y. enterocolitica* strains grown in the defined medium. Vigorous aeration was necessary for the production of maximal levels of YST, and the enterotoxin was not detected in culture supernatants of *Y. enterocolitica* strains grown in the defined medium at  $\geq 30$  degree C.

L44 ANSWER 9 OF 16 MEDLINE on STN DUPLICATE 5  
ACCESSION NUMBER: 93054768 MEDLINE  
DOCUMENT NUMBER: 93054768 PubMed ID: 1429737  
TITLE: Electrophoretic studies on the assembly of the nitrogenase molybdenum-**iron** protein from the *Klebsiella pneumoniae* nifD and nifK gene products.  
AUTHOR: White T C; Harris G S; Orme-Johnson W H  
CORPORATE SOURCE: Department of Chemistry, Massachusetts Institute of Technology, Cambridge 02139.  
CONTRACT NUMBER: GM30943 (NIGMS)  
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1992 Nov 25) 267 (33) 24007-16.  
Journal code: 2985121R. ISSN: 0021-9258.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199212  
ENTRY DATE: Entered STN: 19930122  
Last Updated on STN: 19970203  
Entered Medline: 19921222  
AB The electrophoretic properties of the molybdenum-**iron** (MoFe) protein component of nitrogenase and an **iron**-molybdenum cofactor (FeMoco)-reactivatable apoMoFe protein from *Klebsiella pneumoniae* were examined under anaerobic ( $[O_2] < 5$  ppm), nondenaturing conditions. In wild type *K. pneumoniae* extracts, two immunoreactive species migrating more slowly than purified MoFe protein were detected using anti-MoFe protein antibodies. The uppermost species comigrates with the apoMoFe **protein produced** by a *K. pneumoniae* mutant unable to synthesize FeMoco (UN106) and by *Escherichia coli* harboring the plasmids

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pVL222+pVL15 (nifHDKTYUSWZM+A). In vitro FeMoco titration of the UN106 and pVL222+pVL15 extracts increases the electrophoretic mobility of the apoMoFe protein to that of purified MoFe protein in a two-step process giving rise to a species of intermediate mobility between the apo- and holoMoFe proteins. Two-dimensional gel electrophoresis showed that a 20-kDa peptide is associated with the apoMoFe protein and with the intermediate species, but not with the holoMoFe protein. N-terminal sequencing identified this associated peptide as the nifY gene product, which we propose is acting as a temporary enforcer of the apoMoFe protein structure required for cofactor binding that is released upon FeMoco activation. This FeMoco-induced mobility shift was used to characterize the mutant apoMoFe **proteins produced** in E. coli as a result of deleting the various nitrogen fixation (nif) genes from the plasmid pVL222. E. coli **extracts** bearing plasmids deleted in nifH, nifS, nifTYUM, or nifWZM exhibit less than 10% of the apoMoFe protein activity of derepressed UN106 and contain an immunoreactive species whose electrophoretic mobility is increased upon addition of FeMoco from that of apoMoFe protein to that of holoMoFe protein in a single step. Anaerobic nondenaturing gel electrophoresis of <sup>55</sup>Fe-labeled E. coli **extracts** followed by autoradiography showed that these inactive apoMoFe species do not contain **iron**, indicating that the P-clusters are absent. We therefore propose that NifH, S, U, W, Z, and M are all involved, to varying degrees, in P-cluster assembly. In addition, the presence of the P-clusters does appear to be necessary for the two-step FeMoco activation of the apoMoFe protein to occur.

L44 ANSWER 10 OF 16 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN  
ACCESSION NUMBER: 1990-292251 [39] WPIDS  
DOC. NO. CPI: C1990-126118  
TITLE: Vaccines against septicaemia bacteria - containing  
**iron**-regulated outer-membrane proteins.  
DERWENT CLASS: B04 D16  
INVENTOR(S): AUDONNET, J C; BRUNEAU, P  
PATENT ASSIGNEE(S): (INMR) RHONE MERIEUX SA  
COUNTRY COUNT: 17  
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
EP 389347	A	19900926	(199039)*		
	R:	AT BE CH DE ES FR GB GR IT LI LU NL SE			
WO 9011349	A	19901004	(199042)		
	W:	AU CA US			
FR 2644346	A	19900921	(199045)		
AU 9054153	A	19901022	(199104)		
JP 03143388	A	19910618	(199130)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
EP 389347	A	EP 1990-400734	19900319
FR 2644346	A	FR 1989-3626	19890320
JP 03143388	A	JP 1990-71723	19900320

Searcher : Shears 308-4994

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PRIORITY APPLN. INFO: FR 1989-3626 19890320

AN 1990-292251 [39] WPIDS

AB EP 389347 A UPAB: 19930928

Vaccines against septicaemia bacteria contain antigens comprising:  
(A) bacterial cells obtd. by culturing a bacterium in a medium in which the **Fe** content is reduced to a level resulting in increased **production** of **iron-regulated** outer-membrane **proteins** (IROMPs), including siderophore or transferring receptors, or fragments of such cells, or the IROMPs extracted from such cells; or (B) IROMPs and/or IROMP precursors **extracted** from the outer membrane or cytoplasm of recombinant **bacteria** containing vectors for expression of such proteins. The bacteria are E.coli, Klebsiella spp., Salmonella typhimurium or Shigella spp. The IROMPs are Iut A and/or FepA. A vector for expression of Iut A is obtained by isolating a fragment containing the iut A gene from bacterial plasmid or chromosomal **DNA**, cloning the fragment, and inserting it into pGT1001. A vector for expression of Fep A is obtained by isolating a fragment containing the fep A gene from pMS101, cloning the fragment, and inserting it into pGT1001. In case (A), the **Fe** content i.s. reduced by adding an **Fe**-chelating protein, e.g. lactoferrin. @  
0/0

L44 ANSWER 11 OF 16 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on  
STN DUPLICATE 6

ACCESSION NUMBER: 1990:493196 BIOSIS

DOCUMENT NUMBER: PREV199090121542; BA90:121542

TITLE: IN-VITRO EXPRESSION OF LAC-PTS AND TAGATOSE  
1 6-BISPHOSPHATE ALDOLASE GENES FROM  
LACTOCOCCUS-LACTIS-SSP-CREMORIS PLASMID PDI-21.

AUTHOR(S): YU P-L [Reprint author]; HODGE R A; LI X-P

CORPORATE SOURCE: DEP BIOTECHNOL, MASSEY UNIV, PALMERSTON NORTH, NZ  
SOURCE: Applied Microbiology and Biotechnology, (1990) Vol.  
33, No. 6, pp. 677-679.  
CODEN: AMBIDG. ISSN: 0175-7598.

DOCUMENT TYPE: Article  
(Retraction)  
Errata

FILE SEGMENT: BA

LANGUAGE: ENGLISH

ENTRY DATE: Entered STN: 5 Nov 1990

Last Updated on STN: 5 Nov 1990

AB A 4.4-kb EcoR1-EcoR1 **DNA** fragment from the Lactococcus lactis subsp. cremoris plasmid pDI-21 encoded the tagatose 1,6-bisphosphate (TBP) aldolase gene and the Lac-PTS genes. In vitro transcription-translation using Escherichia coli S30 **extract** showed the synthesis of 41,000-, 23,000- and 12,000-dalton proteins which correspond to the TBP-aldolase, Lac-PTS enzyme II, and factor III proteins respectively.

L44 ANSWER 12 OF 16 MEDLINE on STN

ACCESSION NUMBER: 90174948 MEDLINE

DOCUMENT NUMBER: 90174948 PubMed ID: 2408009

TITLE: Complementation of the xeroderma pigmentosum  
**DNA** repair **synthesis** defect with

Searcher : Shears 308-4994

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Escherichia coli UvrABC **proteins** in a cell-free system.  
AUTHOR: Hansson J; Grossman L; Lindahl T; Wood R D  
CORPORATE SOURCE: Imperial Cancer Research Fund, Clare Hall Laboratories, Herts, UK.  
SOURCE: NUCLEIC ACIDS RESEARCH, (1990 Jan 11) 18 (1) 35-40.  
Journal code: 0411011. ISSN: 0305-1048.  
PUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199004  
ENTRY DATE: Entered STN: 19900601  
Last Updated on STN: 19900601  
Entered Medline: 19900404

AB A newly developed cell-free system was used to study **DNA** repair synthesis carried out by extracts from human cell lines in vitro. Extracts from a normal human lymphoid cell line and from cell lines established from individuals with hereditary dysplastic nevus syndrome perform damage-dependent repair synthesis in plasmid **DNA** treated with cis- or trans-diamminedichloro-**platinum(II)** or irradiated with ultraviolet light. Cell extracts of xeroderma pigmentosum origin (complementation groups A, C, D, and G) are deficient in **DNA** repair synthesis. When damaged plasmid **DNA** was pretreated with purified Escherichia coli UvrABC proteins, xeroderma pigmentosum cell **extracts** were able to carry out **DNA** repair synthesis. The ability of E. coli UvrABC proteins to complement xeroderma pigmentosum cell **extracts** indicates that the extracts are deficient in incision, but can carry out later steps of repair. Thus the in vitro system provides results that are in agreement with the incision defect found from studies of xeroderma pigmentosum cells.

L44 ANSWER 13 OF 16 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 1989:339436 BIOSIS  
DOCUMENT NUMBER: PREV198988042436; BA88:42436  
TITLE: THE BINDING ACTIVITIES OF PROTEINS THAT BIND AP-4A AN ALARMONE ARE STIMULATED IN THE PRESENCE OF ETHANOL OR PHOSPHATIDYLETHANOLAMINE.  
AUTHOR(S): KOBAYASHI Y [Reprint author]; KURATOMI K  
CORPORATE SOURCE: DEP BIOCHEM, TOKYO MED COLL, 6-1-1, SHINJUKU-KU, TOKYO 160, JAPAN  
SOURCE: Biochemical and Biophysical Research Communications, (1989) Vol. 160, No. 3, pp. 1379-1386.  
CODEN: BBRCA9. ISSN: 0006-291X.  
DOCUMENT TYPE: Article  
FILE SEGMENT: BA  
LANGUAGE: ENGLISH  
ENTRY DATE: Entered STN: 20 Jul 1989  
Last Updated on STN: 27 Jul 1989

AB Three proteins binding Ap4A which is known to increase in the heat-shocked cells or to trigger DNA4 synthesis in G1-arrested eukaryotic cells were purified from Escherichia coli cell **extract**. For the binding activities of the proteins, glutathione or dithiothreitol and manganese or **iron** ion were absolutely required. Glutathione, which exists in relatively

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high concentration in the cells and had been reported to be related to oxidant shock, was far more effective than an artificial antioxidant, dithiothreitol. Ethanol, which has an effect similar to heat or oxidant shock on microbial or eukaryotic cells, enhanced several fold the Ap4A-binding activity. Phosphatidylethanolamine, a major component of phospholipids of cytoplasm and membrane of E. coli cell also stimulated the Ap4A-binding activity.

L44 ANSWER 14 OF 16 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN  
ACCESSION NUMBER: 1982-68470E [33] WPIDS  
TITLE: **Peptide** antibiotic with antibacterial  
activity - **produced** by culturing  
Streptomyces sp. WS 116.  
DERWENT CLASS: B03 D16  
INVENTOR(S): BENZ, G; METZGER, K G; PFITZNER, J; SCHMIDT, D;  
SCHROEDER, T; WEYLAND, H  
PATENT ASSIGNEE(S): (FARB) BAYER AG  
COUNTRY COUNT: 12  
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
EP 57349	A	19820811	(198233)*	GE	27
R: AT BE CH DE FR GB IT LI NL SE					
DE 3102137	A	19820819	(198234)		
JP 57143000	A	19820903	(198241)		
US 4415557	A	19831115	(198348)		
EP 57349	B	19840627	(198427)	GE	
R: AT BE CH DE FR GB IT LI NL SE					
DE 3260277	G	19840802	(198432)		
US 4546079	A	19851008	(198543)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
EP 57349	A	EP 1982-100121	19820111
US 4415557	A	US 1983-499653	19830531

PRIORITY APPLN. INFO: DE 1981-3102137 19810123

AN 1982-68470E [33] WPIDS

AB EP 57349 A UPAB: 19930915

A new antibiotic (I), obtainable in **Fe**-containing or **Fe**-free form, is produced by culturing Streptomyces sp. WS116 (DSM 1692) under submerge aerobic conditions in a medium containing C and N sources and mineral salts (especially **Fe** salts) at 15-35 deg.C.

The **Fe**-free form of (I) is characterised by its physico-chemical properties and by the fact that it releases ornithine and serine in 3:1 ratio upon acid hydrolysis. A cpd. of formula (Ia) is specifically claimed: where Z is a gp. of formula (II). WS116 is pref. cultured in a medium containing citric acid as C source. The N source can be L-arginine or a conventional N source containing added L-ornithine and L-serine. (I) is pref. isolated by chromatography on a cation exchanger in ferric form.

(I) has antibacterial activity e.g. with MIC values of 0.2 mcg/ml or less against E.coli, K.pneumoniae, and S.aureus.

Searcher : Shears 308-4994



ABEQ EP 57349 B UPAB: 19930915

New antimicrobial designated component B (see EP--57812-B) and its **Fe(III)** salt has formula (I). It is characterised by (a) decomposition **pt.** 185 deg.C, (b) optical rotation ( $\alpha$ )<sub>20D</sub> - 22.7 deg. ( $c=0.2723$  in water immediately after dissolution), (c) readily soluble in water at pH 7, noticeably in methanol, DMF and DMSO, sparingly soluble in chloroform, ether, ethyl acetate and petroleum ether, (d) uv max. at 304 nm in water (acid), (e) ir adsorption maxima at 3388, 2944, 1695, 1648, 1545, 1457, 1417, 1390, 1299, 1240, 1070, 1050 and 975 as KBr pressed plate, and (f) releases ornithine and serine in the ratio 3:1 on acid hydrolysis.

It is prepd. by submerge, aerobic fermentation of *Streptomyces* spec. WS 116(DSM1692) at 15-35 deg.C, in a nutrient contg. citric acid as the main C-source, several complex N-sources (**yeast extract**, soya bean flour and cornsteep liquor) and **amino acids** L-ornithine and L-serine, and mineral salts.

Component B is used in human and veterinary medicine.

ABEQ US 4546079 A UPAB: 19930915

Antibiotic, **Fe-free** or **Fe-contg.** 'BAY i 3265' cpds. is new. Prodn. process comprises growing aerobically biologically pure *Streptomyces* Sp. WS 116 (DSM 1692), isolated from marine soil, in nutrient media contg. C (citric acid) and N (L-ornithine, L-serine), salts and 0.5-2 (0.1)%  $\text{FeCl}_3$  at Ph 6-9.5 (7.5). The antibiotic is isolated by cation exchange and CN-cellulose chromatography.

USE - At dosage 0.1-100 (2-50) mg/kg in treatment of microbial esp. bacterial diseases (e.Coli), without side effects.

L44 ANSWER 15 OF 16 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 77009431 EMBASE

DOCUMENT NUMBER: 1977009431

TITLE: Mechanism of plasmid mediated resistance to cadmium in *Staphylococcus aureus*.

AUTHOR: Chopra I.

CORPORATE SOURCE: Dept. Bacteriol., Med. Sch., Univ. Bristol, United Kingdom

SOURCE: ANTIMICROB.AGENTS CHEMOTHER., (1975) 7/1 (8-14).

CODEN: AACHAX

DOCUMENT TYPE: Journal

FILE SEGMENT: 037 Drug Literature Index

004 Microbiology

030 Pharmacology

LANGUAGE: English

AB The mechanism of plasmid mediated resistance to cadmium in *S. aureus* was investigated. **Protein synthesis** in cell free **extracts** from resistant or susceptible **bacteria** was equally susceptible to inhibition by  $\text{Cd}^{2+}$ , but spheroplasts from resistant bacteria retained their resistance. Resistant bacteria did not have a decreased affinity for cations in general, nor was active metabolism required for exclusion of  $\text{Cd}^{2+}$ . The kinetics of  $\text{Cd}^{2+}$  uptake into susceptible and resistant bacteria suggested that the conformation of membrane proteins in resistant bacteria may be important in the exclusion of  $\text{Cd}^{2+}$ .

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09/989974

ACCESSION NUMBER: 74186709 EMBASE  
DOCUMENT NUMBER: 1974186709  
TITLE: Synthesis of diphtheria tox gene products in  
Escherichia coli extracts.  
AUTHOR: Murphy J.R.; Pappenheimer Jr A.M.; De Borms S.T.  
CORPORATE SOURCE: Biol. Lab., Harvard Univ., Cambridge, Mass. 02138,  
United States  
SOURCE: Proceedings of the National Academy of Sciences of  
the United States of America, (1974) 71/1 (11-15).  
CODEN: PNASA6  
DOCUMENT TYPE: Journal  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: English

AB In a **protein synthesizing** system  
**extracted** from E. coli, purified **DNA**  
from corynephages  $\beta$ c(tox+) and  $\beta$ 45c was used to direct the  
in vitro synthesis of diphtheria toxin and of the related nontoxic  
protein, CRM45, as well as of other  $\beta$  phage proteins. When  
 $\beta$ c(tox+) **DNA** or  $\beta$ 45c **DNA** was added to  
a similar system extracted from the non lysogenic C. diphtheriae  
strain, C7(s)(-)tox-, neither toxin nor the CRM45 **protein**  
was **produced**, although other  $\beta$  phage **proteins**  
were **synthesized** in amounts equivalent to those produced  
in the E. coli system from the same amount of  $\beta$  phage  
**DNA**. Preliminary experiments suggest that both toxinogenic  
and nontoxinogenic strains of the diphtheria bacillus contain a  
factor that specifically blocks expression of the tox gene.  
**Synthesis** of toxin and the CRM45 **protein** in the E.  
coli system could not be inhibited by relatively C7(s)(-)tox- high  
concentrations of inorganic **iron**, but could be inhibited  
by extracts from the strain of C. diphtheriae.

(FILE 'MEDLINE' ENTERED AT 15:56:53 ON 24 OCT 2003)

L20 120010 SEA FILE=MEDLINE ABB=ON PLU=ON PROTEINS/CT  
L22 155049 SEA FILE=MEDLINE ABB=ON PLU=ON "ESCHERICHIA COLI"/CT  
L23 8788 SEA FILE=MEDLINE ABB=ON PLU=ON YEASTS/CT  
L24 55770 SEA FILE=MEDLINE ABB=ON PLU=ON BACTERIA/CT  
L48 78998 SEA FILE=MEDLINE ABB=ON PLU=ON (MERCURY OR PLATINUM OR  
IODINE OR IRON OR SELENIUM)/CT  
L49 1425 SEA FILE=MEDLINE ABB=ON PLU=ON L48 AND L20  
L50 89613 SEA FILE=MEDLINE ABB=ON PLU=ON "AMINO ACIDS"/CT  
L51 118 SEA FILE=MEDLINE ABB=ON PLU=ON L49 AND L50  
L52 6 SEA FILE=MEDLINE ABB=ON PLU=ON L51 AND (L22 OR L23 OR  
L24)

L53 6 L52 NOT L25

L53 ANSWER 1 OF 6 MEDLINE on STN  
AN 96165508 MEDLINE  
TI A new selenoprotein from human lung adenocarcinoma cells:  
purification, properties, and thioredoxin reductase activity.  
AU Tamura T; Stadtman T C  
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES  
OF AMERICA, (1996 Feb 6) 93 (3) 1006-11.  
Journal code: 7505876. ISSN: 0027-8424.  
AB We report the isolation and characterization of a new selenoprotein  
from a human lung adenocarcinoma cell line, NCI-H441. Cells were

Searcher : Shears 308-4994

grown in RPMI-1640 medium containing 10% (vol/vol) fetal bovine serum and 0.1 microM [75Se]selenite. A 75Se-labeled protein was isolated from sonic extracts of the cells by chromatography on DE-23, phenyl-Sepharose, heparin-agarose, and butyl-Sepharose. The protein, a homodimer of 57-kDa subunits, was shown to contain selenium in the form of selenocysteine; hydrolysis of the protein alkylated with either iodoacetate or 3-bromopropionate yielded Se-carboxymethyl-selenocysteine or Se-carboxyethyl-selenocysteine, respectively. The selenoprotein showed two isoelectric points at pH 5.2 and pH 5.3. It was distinguished from selenoprotein P by N-glycosidase assay and by the periodate-dansylhydrazine test, which indicated no detectable amounts of glycosyl groups on the protein. The selenoprotein contains FAD as a prosthetic group and catalyzes NADPH-dependent reduction of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), and reduction of insulin in the presence of thioredoxin (Trx). The specific activity was determined to be 31 units/mg by DTNB assay. Apparent Km values for DTNB, *Escherichia coli* Trx, and rat Trx were 116, 34, and 3.7 microM, respectively. DTNB reduction was inhibited by 0.2 mM arsenite. Although the subunit composition and catalytic properties are similar to those of mammalian thioredoxin reductase (TR), the human lung selenoprotein failed to react with anti-rat liver TR polyclonal antibody in immunoblot assays. The selenocysteine-containing TR from the adenocarcinoma cells may be a variant form distinct from rat liver TR.

- L53 ANSWER 2 OF 6 MEDLINE on STN  
 AN 90160870 MEDLINE  
 TI Amplification of bacterial plasmids without blocking protein biosynthesis.  
 AU Angelov I; Ivanov I  
 SO PLASMID, (1989 Sep) 22 (2) 160-2.  
 Journal code: 7802221. ISSN: 0147-619X.  
 AB The effect of amino acids (presence or absence from the growth media) and metal ions on the replication of *Escherichia coli* plasmids in rel A+ strains was studied. It was found that: (i) The absence of one amino acid from the growth media had no effect on the plasmid copy number in prototrophic *E. coli* strains; (ii) The presence of only one amino acid in artificial media free of amino acids had a negligible effect on the plasmid copy number for the amino acids Ala, Arg, Glu, His, Leu, Phe, Thr, Trp, and Tyr; (iii) The combination of Met and Thr caused a rise in pBR322 plasmid copy number up to 90-100 plasmid copies per cell; (iv) The Fe3+ concentration had an amplification effect on *E. coli* plasmids. The pBR322 plasmid copy number for media free of amino acids and supplemented with 0.2-0.4 mM FeCl3 was 60-80 plasmid copies per cell; (v) The combination of Fe3+ with certain amino acids (Ala, Arg, Glu, Leu, Thr, and Trp) leads to a dramatic increase in the plasmid copy number reaching 180-270 plasmid copies per cell for the plasmid pBR322 and 20-24 for the plasmid pR100.
- L53 ANSWER 3 OF 6 MEDLINE on STN  
 AN 83003654 MEDLINE  
 TI A novel non-heme iron-containing dioxygenase. Chloridazon-catechol dioxygenase from *Phenylobacterium immobilis* DSM 1986.  
 AU Muller R; Schmitt S; Lingens F  
 SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (1982 Jul) 125 (3) 579-84.  
 Journal code: 0107600. ISSN: 0014-2956.  
 AB Previously we purified an enzyme from *Phenylobacterium immobilis* DSM

1986, which cleaves the catechol derivative of the herbicide Chloridazon [5-amino-4-chloro-2-phenyl-3 (2H)-pyridazinone] in the meta position. The enzyme, which could be crystallized, proved in Ouchterlony double-diffusion tests to consist of a single protein species. No cross-reaction was observed with other meta-cleaving enzymes. Its light absorption spectrum showed a maximum at 279 nm ( $\epsilon = 310 \text{ mM}^{-1} \text{ cm}^{-1}$ ), shoulders at 289 nm and 275 nm and a very weak band at around 430 nm ( $\epsilon = 1.14 \text{ mM}^{-1} \text{ cm}^{-1}$ ). The amino acid analysis showed a slight excess of acidic amino acids, in agreement with the pI of 4.5. Surprisingly the enzyme per se is completely inactive, although it contains one non-dialysable iron atom per submit. It has to be activated by preincubation with ferrous ions or ascorbate. The enzyme activated this way is autoxidizable and returns to its non-activated state in the presence of oxygen. During the reaction with the substrate, this inactivation seems to be enhanced about 100 times. Since this kind of activation and inactivation is not observed in other meta-cleaving enzymes, this enzyme seems to represent a new type of a non-heme iron dioxygenase. We tentatively propose the name Chloridazon-catechol dioxygenase for this enzyme.

- L53 ANSWER 4 OF 6 MEDLINE on STN  
 AN 80020161 MEDLINE  
 TI Studies on the inhibition of protein synthesis by selenodiglutathione.  
 AU Vernie L N; Collard J G; Eker A P; de Wildt A; Wilders I T  
 SO BIOCHEMICAL JOURNAL, (1979 Apr 15) 180 (1) 213-8.  
 Journal code: 2984726R. ISSN: 0264-6021.
- AB Amino acid incorporation in a cell-free system derived from rat liver has previously been found to be inhibited by GSSeSG (selenodiglutathione). In the present experiments the effect of GSSeSG on protein synthesis in 3T3-f cells, on growth and protein synthesis in Escherichia coli, and on amino acid incorporation in a cell-free system derived from E. coli, was studied. GSSeSG inhibits the incorporation of [3H]leucine into protein by 3T3-f cells. This inhibition cannot be reversed by removing GSSeSG and is correlated with the uptake of GSSeSG. Sodium selenite ( $\text{Na}_2\text{SeO}_3$ ) and oxidized glutathione had no inhibitory effect in this system. [3H]Uridine or [3H]thymidine incorporation into RNA or DNA was not inhibited, indicating that the primary action of GSSeSG was on protein synthesis. GSSeSG did not influence the growth of E. coli in a synthetic medium, although enhanced amino acid incorporation was observed. In the cell-free system derived from E. coli, amino acid incorporation was not changed by GSSeSG, indicating that elongation factor G, in contrast to elongation factor 2 of mammalian cell systems, is not blocked by GSSeSG.
- L53 ANSWER 5 OF 6 MEDLINE on STN  
 AN 73141133 MEDLINE  
 TI Biochemical effects of mercury, cadmium, and lead.  
 AU Vallee B L; Ulmer D D  
 SO ANNUAL REVIEW OF BIOCHEMISTRY, (1972) 41 (10) 91-128. Ref: 594  
 Journal code: 2985150R. ISSN: 0066-4154.
- L53 ANSWER 6 OF 6 MEDLINE on STN  
 AN 69060565 MEDLINE  
 TI Toxic amino acids: their action as antimetabolites.  
 AU Fowden L; Lewis D; Tristram H

09/989974

SO ADVANCES IN ENZYMOLOGY AND RELATED AREAS OF MOLECULAR BIOLOGY,  
(1967) 29 89-163. Ref: 248  
Journal code: 0337243. ISSN: 0065-258X.

FILE 'HOME' ENTERED AT 15:58:18 ON 24 OCT 2003